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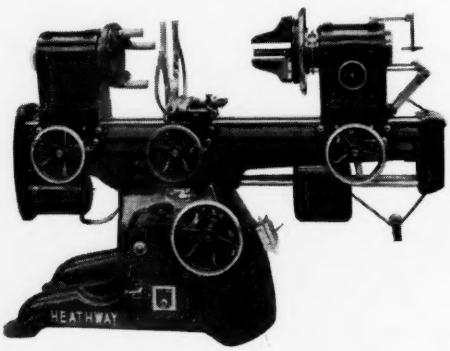

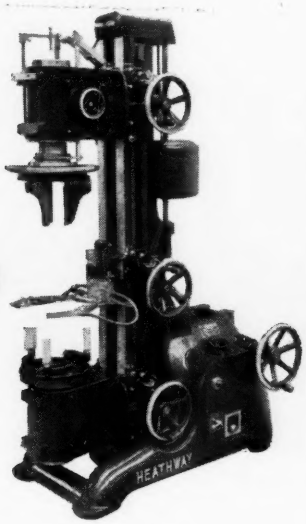

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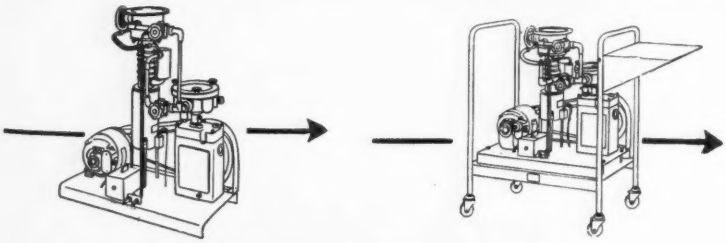

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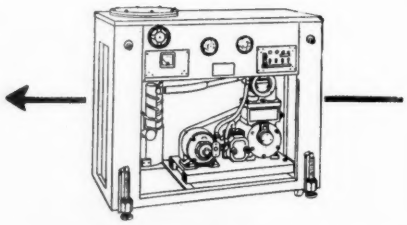

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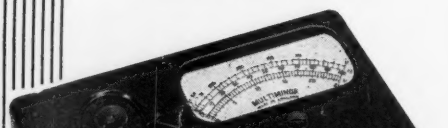

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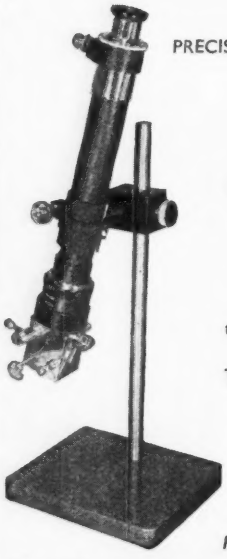

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APPLICATION OF SCIENCE IN INDUSTRY

WITH their third report, written for the Science and Industry Committee set up by the British Association in 1952 and re-constituted in 1954 with the additional sponsorship of the Royal Society of Arts and the Nuffield Foundation, Prof. C. F. Carter and B. R. Williams complete the greater part of their work on the terms of reference of that Committee*. Their analysis of, and broad conclusions on, the factors governing the speed of application of science were reported in their first book, "Industry and Technical Progress", in 1957. In a second volume, in 1958, "Investment in Innovation", some of the earlier material was re-examined to elucidate factors which influence industrial investment in new plant and equipment. Some of that material is again discussed in this concluding volume, which is concerned to outline a policy for action.

The proposals set forth are grouped broadly according to whether the action required is by industry or by Government, and at the present moment, in view of the recent establishment of a Minister for Science, the second part of the book, in which various important aspects of public policy are examined, is of special interest. The reader is left in no doubt that some conditions of technical progress are created by Government action, though he may be surprised at the limitations of existing knowledge as to the extent and effect of such action. There is still, in fact, a very considerable field calling for further inquiry, especially perhaps on the financial side, as was particularly emphasized in "Investment in Innovation". On numerous points the facts on which public policy should be based have still to be determined, and while, like its predecessors, this third volume should stimulate as well as guide thought, there is nothing in it to urge hasty decision or action upon Lord Hailsham as Minister for Science.

Profs. Carter and Williams begin their consideration of the instruments of policy by which the Government can support and assist technical progress with a broad discussion of the strategy of Government aid—a discussion which is surely as broad as Lord Hailsham himself or Sir Hugh Beaver has already given, although it touches on some matters of Government policy directed at other objectives which may incidentally affect the advancement of technique. This Government help may be provided by many different departments and State-sponsored bodies; for example, the National Research Development Corporation. In some Departments, however, affairs may be involved in such instruments as purchase tax, which may incidentally hinder the adoption of new techniques. Without entering on any analysis of the actual organization for Government assistance to research and development even, to the extent to be found in the survey made by a study group of the Royal Institute of Public Administration and published in 1957 in "The Organization of British Government", Profs. Carter and Williams suggest that some re-assessment is needed of Government aid to research and development, designed to make it more likely that it will give help to industry at the points where, in the long run, help is most needed.

This re-assessment would require separate consideration of the needs for advice, for assistance to research and for assistance to development, and at present Profs. Carter and Williams doubt if it can be said that a Government policy on the application of science really exists. The facts on which such a policy should be based, they assert, have never been collected or assessed. Substantial research is needed, and the problem is both technical and economic, requiring extensive and continued study by a group containing both scientists and economists. They do not think that such a group exists at present, but without it policy-making tends to go no further than a statement of generalities.

It appears to be to the Advisory Council on Scientific Policy that the Science and Industry Committee looks for progress here, although it is implicit in what is written in the present report that the Advisory Council has been, particularly of recent years, too preoccupied with comparatively minor matters. It is believed that Lord Hailsham intends to make increasing use of the Advisory Council, and he may well look to the Council for help in seeking answers to some of the more fundamental questions, such as the proper place and the right limit for Government action in speeding the application of science, the scale of Government effort, the extent to which it should be concentrated or widely diffused and, if concentrated, the points of maximum effort, to which answers must be sought and found before a national policy is formulated.

Besides this emphasis on the need for much more patient fact-finding, stress is laid on the importance of 'cost- and profit-mindedness' in planning national research policy, as in planning research in an individual firm, and this aspect must be given due weight in making the inevitable choice between fields of effort. It is duly noted that the Council for Scientific and Industrial Research appears to have this question of priorities very much in mind, but it is also clear that the Science and Industry Committee is somewhat reserved as to whether sound decisions can yet be made. The report, in fact, goes no further than to suggest a few principles.

The Committee hesitated, for example, to recommend more than occasional use of the policy of 'reinforcing success', helping industries which are

* "Science in Industry: Policy for Progress". By C. F. Carter and B. R. Williams. Pp. ix+186. (London: Oxford University Press, 1959.) 21s. net.

already showing signs of lively innovation to advance still faster, although the principle deserves examination. On the other hand, these studies support the policy of concentration at weak points, not indeed to the extent of helping weakness wherever it is found, but rather of helping industries unlikely to achieve rapid technical progress with their own resources, and especially those industries which have great promise of change or development. There is no support for the practice, too common already in British industry, of bolstering the inefficient.

Such a policy is not easy to apply, but obviously it is one which must be carefully considered in determining State policy towards the research associations in Britain. Here the further suggestion is made that assistance for advisory services, including educational services, should be provided separately from that for research and specifically earmarked. Such a step would have certain advantages in assessing the magnitude of the national effort in research, and the recognition of the educational work of the research associations is in keeping with the view that the Government's fundamental contribution to technical progress is in its support of training facilities. Beyond that the question of balance between research and development is all-important.

In this matter of development, some extension of the practice of establishing development companies by the research associations is recommended, as well as extension of sponsored research facilities. The studies made for the Science and Industry Committee suggest, however, that existing facilities for the finance of development through the National Research Development Corporation are not fully used, and that Government support may need to go beyond the financing of development contracts. While Government Departments as well as research associations should make full use of the experience and funds of that Corporation, it may occasionally be desirable to create State development companies to manage development in a defined field. Nevertheless, every effort should be used to make the National Research Development Corporation an effective instrument of development on behalf of all Government-aided research bodies which cannot achieve adequate development from their own resources, and its powers to stimulate research (as well as development) should be used with vigour to fill in gaps which may appear in the structure of industrial research, or to recommend action for filling them to the national research councils.

On this view there is a strong case for placing the National Research Development Corporation within the authority of the Minister for Science, and probably within the responsibility of the Advisory Council on Scientific Policy. Its responsibility to the Board of Trade is obviously anomalous, and although Profs. Carter and Williams do not commit themselves as to whether they regard the Corporation as sufficiently in direct touch with the economic as well as the technical problems of industry, they are in no doubt as to the value of its present work. No evidence is presented as to the advantage which any new type of body would offer in assessing Government aid to industry as a problem in the joint use of economics and technology.

On educational policy the report makes numerous sound and sensible comments, few of which are new, but some of which are still badly needing attention. Such, for example, are the reminder of the waste of highly trained man-power which follows from an insufficient supply of technicians, and the clear warning that, unless corrective measures are taken immediately, a swift rise in the numbers of scientists and technologists available in the mid-1960's will be unmatched by an equivalent rise in the number of technicians and craftsmen, and the existing lack of balance will be made worse. Again, while no evidence was found that industry in general is failing to get its fair share of first-rate men, the inquiry pointed to the desirability of further study of the cost of additional years of education in relation to the benefits to be expected. Here the evidence indicates that a higher scientific content in the education of most citizens would promote technical progress, partly by removing the obstacle which 'scientific illiteracy' offers to the communication of ideas, and partly because a greater reserve of people with some grounding in science would assist industry to meet demands, perhaps unexpected, for new types of technical skill.

There are also sound comments on the importance of increased training for craftsmen and apprentices, increased attention to the education of technicians, and, by the universities, to the finding of the best form of education for managers; and the bearing of a better provision for laboratory technicians in schools on the supply of science teachers is duly noted. Some of the points made in regard to the supply of science teachers have already been considered by the Advisory Council on Scientific Policy, but they are presented here in a way which shows very clearly their direct bearing on the responsibilities now entrusted to Lord Hailsham. The educational implications of his task could not easily be summarized better, and he can scarcely overlook the emphatic suggestion that effective research in the use made of scientists and technologists would make the planning of educational facilities much more reliable.

On taxation policy the report does not add substantially to the conclusions outlined in "Industry and Technical Progress"; and it gives little support to the view that high taxation discourages technical advance, although it concedes that it could do so. It urges that it is difficult to devise reductions in taxation which will stimulate technical progress without also giving large benefits to firms in which progress is unaffected by taxation, or to firms which would be unprogressive whatever the level of taxation. A lowering and equalization of taxes on expenditure, and a reduction in surtax rates would, however, probably favour technical progress, and when increases of taxes are necessary, widely spread increases are least likely to affect technical progress. What matters most, however, is that changes in

taxation should be as infrequent as possible; the Committee's studies confirm the conclusion that the uncertainty created by such changes and the expectation of tax changes have an unsettling effect on planning technical advance, and while the report recommends specifically the consideration of special investment allowances for development costs and also of a scheme by which firms would be allowed to write off their assets for tax purposes at any rate they chose, subject to adoption of the same rate in their accounts, these suggestions are subject to that over-riding consideration.

Here again the importance of Lord Hailsham's recent appointment is evident. If this consideration is to be given due weight at Cabinet level, it is important that the implications should be put to the Cabinet by a Minister whose responsibilities for scientific and technical progress are as unequivocal as are those of the Chancellor of the Exchequer in the financial field. Moreover, there are other proposals advanced regarding the effect of taxation on technical progress which have far-reaching political implications, and can accordingly only be considered at a level where those political considerations can also be given due weight. Likewise, the report rejects the idea that there is any general principle that protection favours (or retards) technical progress, and doubts are expressed as to the wisdom of abolishing independent review by the Import Duties Advisory Committee in applying the Import Duties Act, and as to whether issues of technical progress will be of regular substantial importance in arguing cases before the Restrictive Practices Court. No changes are recommended in the legislation against restrictive trading agreements and monopolies, but strong support is lent to all that has been urged elsewhere as to the importance of management giving more attention to overcoming the fear of redundancy. This is the most hopeful means of meeting the restrictive practices of labour, which do occasional damage to technical progress.

So far as can be judged, therefore, the recommendations of the report are in line with the current trend of opinion in Britain and with much evidence that Lord Hailsham has now before him. He has already, for example, declared his intention of strengthening the voice of the Advisory Council on Scientific Policy, though what is most wanted here is the authority which would ensure that due heed is paid to the findings and recommendations of that Council. The stress once again laid on the importance of provision for research and technical education should also encourage proposals already in train, and, no less important, direct fresh attention to the question of balance and phasing. Lastly, the emphatic opinion expressed as to the serious handicap which insecurity of capital programmes can impose on technical progress is valid far more widely than in the nationalized industries, on which it is primarily based, and with a Minister for Science there is perhaps a better prospect of representations being made at a high enough level and an early enough stage to check excessive change.

THE HISTORY OF WESTERN THOUGHT

Wisdom of the West

By Bertrand Russell. Edited by Paul Foulkes. Pp. 320. (London: Macdonald and Co. (Publishers), Ltd., 1959.) 63*s*. net.

THIS is Lord Russell's second brilliant venture into a comprehensive history of Western (mainly philosophical) thought. Again there is the freshness of style, the attempt to relate scientific, logical and sociological issues, though it should be said that, as in the earlier work, the selection of material on the whole is conventional. Little attempt has been made, for example, to include more recent research into the medieval foundations of scientific thought; or again, if the relations of science and seventeenth-century philosophy are touched upon, the philosophical contributions of later centuries are given rather more in isolation from contemporary scientific currents. None the less, the range is encyclopædic, stretching from the glory of Athens (which comprises almost one half the book) to Frege, Bergson, Freud, Wittgenstein, Jaspers and Sartre.

The most fascinating aspect of the new book, however, is its illustrations: historical sites and the great thinkers of the past mingle with reproductions of historical scientific apparatus, and these again with diagrams meant to illustrate the trend of an abstract argument; the whole capped by a number of compositions by John Piper: an exciting excursion into the field of 'visual aids'. No brief review could do justice to the mere pleasure with which the interested reader will turn over the pages, to be dazzled by the variety of the contents.

Of course, despite the brilliance and apparent novelty of presentation, it should be realized that on the whole this is old wine poured into new bottles. To be sure, many of the harsher value judgments of Russell's past writings have been toned down, and there is a generous inclusion of views with which Russell may not always have sympathized, like those of Vico, Bergson, Kierkegaard and many others. But there are still the rough and ready divisions into the Continental rationalists (who are rather wicked) and the more virtuous British empiricists, supported arbitrarily by the quite unproved suggestion that the diagrams representing these two types of philosophical systems are, respectively, a pyramid standing on its head, as against the other, standing on its feet (p. 219). If anything is true, it is that *any* theory of knowledge which claims to be based on a specific limited 'basis' on which all the rest is said to depend (whatever the nature of the basis) should be represented by a pyramid standing on its head: which is an example of the general criticism that the apparent plausibility of a diagram representing an abstract argument could be a dangerous tool in the hands of an unscrupulous teacher. Bacon, as usual, is charged with completely ignoring deductive procedures, the use of hypothesis, and the notion of an underlying 'invisible' structure of matter, though so far as the evidence goes it is more likely that this was a matter of emphasis rather than omission. Peirce (late nineteenth century) is credited with the foundation of the hypothetico-deductive method, a statement which only emphasizes the (usual) omission of earlier writers such as Whewell and Jevons, not to mention three centuries of medieval contributions to the

subject. Kant's thing-in-itself is made a condition of inter-subjective experience (p. 241), which is (to say the least) a misleading appraisal; Mill's immensely subtle argument about the foundations of inductive reasoning is dismissed with the usual hackneyed remark that it is 'circular'. These examples (though they could be argued) suggest that despite the undoubted sharpness and accuracy of most of the accounts to be found here, the author's authority is no substitute for caution on the part of the reader, particularly when it is remembered that the best of the histories of philosophy are those that are deeply felt and take up a certain position. This becomes obvious when the discussion centres around the more recent of Russell's philosophical interests, such as pragmaticist theories of truth, when the argument, though still as lucid as ever, becomes rather more heated.

But these are minor criticisms. Certainly this new excursion into our cultural heritage is another astonishing venture testifying to the brilliance and almost legendary energy of its author.

GERD BUCHDAHL

A HISTORY OF IRON AND STEEL IN CHINA

The Development of Iron and Steel Technology in China

By Dr. Joseph Needham. (Second Biennial Dickinson Memorial Lecture to the Newcomen Society, 1956.) Pp. xii+76+31 plates. (London: The Newcomen Society for the Study of the History of Engineering and Technology, 1958.) 57*s*.; 8.15 dollars.

THIS book, an expansion of a lecture founded in memory of Dr. H. W. Dickinson, who was educated at the Manchester Grammar School and at Owens College, is beyond doubt a first-rate contribution to scholarship. In the field of Chinese science and technology the author speaks with a unique authority based on both wide and deep knowledge.

That China had an ancient and, for the times, an advanced metallurgical industry has long been known, but isolated as it was, the exact nature and chronology of this have been largely matters of conjecture. So far as iron and steel are concerned, we now know how and when it developed, and a most astonishing story it is. Although in Europe it was the end of the fifteenth century A.D. before molten cast iron was first produced except by accident, Dr. Needham adduces the most cogent evidence to show that this was being done in China on a by no means insignificant scale two thousand years before. How this was done, the product obtained, and the probable reasons —the existence of ores high in phosphorus producing highly phosphoric irons of increased fusibility and the invention of really effective double-acting bellows —are all referred back to the literary sources and illustrated. The fifty-odd illustrations, ranging from cast iron moulds of the fourth century B.C. to photomicrographs illustrating modern investigations of the processes described, are all of great interest and value.

From the second century B.C., if not before, this cast iron was used to produce steel by a most carefully controlled decarburization in an air blast, the method of the 'hundred refinings'. Rather later, much of the Chinese steel was made by a 'co-fusion' technique in which wrought and cast iron were heated together in crucibles, the pasty lumps of the former being bathed in the high-carbon liquid. By a gradual diffusion of carbon a steel of approximately eutectoid composition can be produced. In connexion with this production of steel one is inevitably reminded of the work of Réaumur of the very early eighteenth century A.D. The basic correspondence of these processes with those of Huntsman, Bessemer and Siemens and Martin is clearly very much in the mind of Dr. Needham. Indeed, so far as the Bessemer process is concerned, he points out that immediately before the work of William Kelly in the United States, four Chinese workers were imported as experts at his furnaces at Kuttawa.

Many other topics are discussed, from magic swords and human sacrifices to assist the furnace produce the required product, to early Chinese tilting furnaces, and the abundant footnotes are often a sheer delight.

This book on the development of the iron and steel industry has a special interest in view of the present-day small-scale production of iron in China, often by processes which cannot differ essentially from those here described. We must now await even more impatiently a corresponding treatment of the non-ferrous metals.

F. C. THOMPSON

USE OF ISOTOPES IN ORGANIC SYNTHESES

Organic Syntheses with Isotopes

By Arthur Murray, III, and D. Lloyd Williams. Part 2: Organic Compounds labelled with Isotopes of the Halogens, Hydrogen, Nitrogen, Oxygen, Phosphorus, and Sulfur. Pp. ix+1147–2096. (New York: Interscience Publishers, Inc.; London: Interscience Publishers, Ltd., 1958.) 188*s*.

MURRAY and Williams have produced a valuable contribution in this second part of their compilation of organic syntheses involving the use of isotopes. While Part I dealt with the synthesis of nearly 500 compounds labelled with carbon-13 and carbon-14, Part 2 is devoted to a similar treatment of syntheses involving the isotopes of 8 other elements, compounds of bromine, chlorine, iodine, nitrogen-15, oxygen-18, phosphorus-32, sulphur-35, deuterium and tritium occupying 30, 15, 65, 151, 33, 30, 82, 400, and 54 pages respectively. The book provides full practical details, in a format rather like that employed in "Organic Syntheses", for the preparation of several hundred individual compounds. Each preparation is headed by the reaction scheme laid out with full structural formulæ, together with the reference from which it has been abstracted. Then follows the "Procedure", a section of explanatory "Notes" and finally "Other Preparations" in which the literature dealing with the preparation of the compound is briefly reviewed.

The compounds are classified under the element concerned and sub-classified according to the chief functional group, for example, acids and acid derivatives, amines, heterocylics, hydrocarbons, etc. With this arrangement rapid scanning of particular sections is feasible, and the book thus provides a ready source for the chemist searching not only for possible recipes

to prepare isotopically labelled compounds but also for high-efficiency, small-scale syntheses for 'unlabelled' compounds. The largest single section is that describing the preparation of compounds containing deuterium, which is in itself a useful collection of experiments in view of the current interest in mechanistic studies. The sixty or so pages of tables listing the hydrogen-deuterium exchange data culled from the literature are most valuable. The less extensive exchange data for other isotopes are also presented in tabular form. Tables of physical constants for isotopically labelled compounds are given where appropriate.

It has evidently been decided that the reader must buy Parts 1 and 2 and use them in conjunction, for the joint index is in Part 2 and the "Introduction" is in Part I. This is doubly inconvenient and seems false economy, as the inclusion of a repeat introduction and separate index could surely not have added much to the cost of these already expensive volumes. The "Introduction" (30 pages) in Part I explained the layout and scope, the new system of isotopic nomenclature used, the special experimental techniques employed, and has short sections on several other important topics. It is annoying to have constantly to refer to Part I for this information. Apart from this question and some minor errors, for example, on p. 1455, ethynyl benzene is consistently referred to as vinyl benzene, this book is an excellent one which should be invaluable both in the library and the laboratory. It should have something of value for all chemists.

G. EGLINTON

PHARMACEUTICAL RESEARCH IN THE U.S.S.R.

Soviet Pharmaceutical Research

Vol. 1: Pharmaceutical Chemistry. Pp. ii+447. 95 dollars. Vol. 2: Pharmacognosy. Pp. ii+401. 90 dollars. Vol. 3: Medicinal Chemistry. Pp. 556. 100 dollars. (New York: Consultants Bureau, Inc.).

RESEARCH into subjects of a pharmaceutical interest is being actively pursued in the U.S.S.R., but communication, due to language difficulties, is virtually non-existent. Workers in this and allied fields will appreciate the enterprise of those responsible for the three-volume translation into English of "Soviet Pharmaceutical Research". Most of this work seems to have been originally published in the U.S.S.R. during the period 1949–1953, but nevertheless it contains much of interest to present trends in pharmacy generally.

Being an American-inspired translation, British readers should not be misled by the title of Vol. 1, "Pharmaceutical Chemistry". This would be better entitled "Physical Chemistry applied to Pharmaceutics", since it contains reports on 74 research papers covering such topics as solubility, stability, ion exchange, emulsions, suspensions and gels, as well as such diverse topics as conditions for preparing crystalline glucose and an investigation of the causes of granulation of powders. Subjects worthy of note in this volume are the influence of organic additives on crystal solubility; patterns of solubility change; the thermal stability of collagen in the dry state; and the decomposition of the binary compound of glucose with sodium chloride. There are interesting investigations into several aspects of emulsion technology utilizing gelatin and also a classification of pigments by their behaviour in suspension. In the analytical field, papers describe the application of infra-red absorption spectra to an investigation of the intermediate products of vitamin A and carotene syntheses.

In Vol. 2, entitled "Pharmacognosy", the papers are merely classified under the headings alkaloids, oils and glycosides, there being no attempt to provide any further subdivision. Provided that the publishers' selection of material is representative of pharmaceutical research being undertaken in the U.S.S.R., it would appear that the isolation of new alkaloids, their proof of structure and possible synthesis are the main preoccupations. No work is included on the chemical constituents of well-known drugs with the exception of some studies on the alkaloids of aconite. Chemical investigations are reported on the alkaloids of *Delphinium*, *Senecio*, *Orobanche* and *Leontice* as well as on the alkaloids of lesser known plants. Some work is reported on the chemical analysis of a few little known essential oils, and there is a paper on the polarographic estimation of some cardiac glucosides, including strophanthidin.

Vol. 3, "Medicinal Chemistry", consists of translations of 91 original research papers on various aspects of this field of chemistry. One group of papers deals with the structure and activity relationships in antimalarials of the quinoline and acridine series. One paper deals with the production of nicotinic acid from the heavy bases in coal tar (236·5–247° C. fraction), and the yield of commercial nicotinic acid is stated to be in the region of 25–35 per cent of the fraction employed. Syntheses of several anti-tubercular drugs like opianic acid derivative of isonicotinic acid hydrazide and several homologs of *p*-aminosalicylic acid have appeared for the first time. The syntheses of several alkaloids like papavarine and emetine have been elegantly described. The synthesis of compounds with antithyroid activity and the preparation of amino-alcohols similar to ephedrine are presented in two papers which will attract the attention of synthetic chemists. The synthetic work in the field of anthelmintics of the coumarin series, and the investigations into the reactions of mercapto-amino-acids will be appreciated by those who are engaged in this type of work. The work on the synthetic studies of methylene-*bis*-5-alkylbarbituric and *spiro*barbituric acids has met with partial success.

The synthetic work in the field of amino-acids such as the synthesis of B-alanine, tagged ^{35}S-methionine and ^{14}N-sulphanilamidolysine, will attract the attention of biochemists. The research work carried out in the chemistry of chloromycetin analogues should be noticed, as the compounds prepared have been found to be active. There are three papers which describe the structural chemistry of gramicidin *C*. A theoretical paper by Nazarov deals with the nomenclature of synthetic steroids and related substances.

In general, this compendium of translations of selected papers from Soviet chemical and pharmaceutical journals gives a fairly good idea of the modern trends of pharmaceutical research in that country. The editors and the publishers of the volumes deserve thanks for compiling and reproducing this mine of information, even at the moderately high price, for those connected with pharmaceutical research.

D. TRAIN

Theoretical Physics

Thermodynamics, Electromagnetism, Waves, and Particles. By Prof. F. Woodbridge Constant. (Addison-Wesley Physics Series.) Pp. xiii+364. (Reading, Mass.: Addison-Wesley Publishing Company, Inc.; London: Academic Books, Ltd., 1958.) 64*s*.

THIS excellent book is the second of two volumes on theoretical physics by Prof. Constant. The first volume treats mechanics and vector analysis and the second, thermodynamics, electromagnetism, waves and particles. Vector methods are used throughout the second volume and frequent references to the earlier volume are made. For these reasons, it is probably best to use both books together. The vector equations in both volumes make use of the operator ∇ only, the shorthand terms grad, div and curl not being used. The main disadvantage of the second volume for students in the honours schools of physics in English universities is that it does not go quite far enough; the level reached approximating to two-thirds of the way along an honours course. On the other hand, students taking a general science degree would find the approach rather sophisticated as such students are not usually brought up to the use of vector algebra. It would appear, therefore, that such an expensive text will not find favour with students at English universities. Nevertheless the book is well planned and the consistent use of vectors seems to make for a logical presentation. It is a book that will probably be used more by teachers of physics than by students of physics in Britain. Text and diagrams are admirably clear and the binding is excellent. Examples are given at the end of every chapter and answers to alternate questions provided.

R. M. Tennent

High Speed Computing

Methods and Applications. By Dr. S. H. Hollingdale. (Applied Physics Guides.) Pp. xii+244. (London: English Universities Press, Ltd., 1959.) 25*s*. net.

IN recent years the automatic digital computer has become available to a wide field of potential users, either directly or through the agency of various computing services. It is to these users rather than to the computer engineer that Dr. Hollingdale's book is addressed.

After a brief introduction to the binary system and to the representation of numbers in a computer, the author devotes nearly one-half of the book to the problem of how a computer obeys a programme. This theme is elaborated with special reference to *Edsac* and to *Deuce*, which are examples of a typical single-address machine and a rather less typical multi-address machine. The circuit techniques of digital computing are confined to two short chapters on storage devices and the logical design of computing circuits.

It is the final chapters of this book that many will find of greatest interest. A valuable chapter on the operation of a computing service, written mainly from the author's experience of such a service at the Royal Aircraft Establishment, is followed by four chapters on applications. In addition to general topics, these cover the Monte Carlo method, the control of industrial processes and the machine translation of languages.

This book is thus in the main concerned with the operation of machines as a whole rather than of their component parts, while the mathematics assumed is not above ordinary level of the General Certificate Examination. This approach provides an ideal introduction to the subject for potential user and non-specialist alike.

E. M. Deeley

The Chemistry of Vegetable Tannins

A Symposium. (Being the Papers presented at a Symposium held in the University, Cambridge, on April 12th–13th, 1956, under the auspices of the Society of Leather Trades' Chemists.) Pp. 160. (Croydon: Society of Leather Trades' Chemists, 1956.) 30*s*. net.

IN view of the efforts made in the last decade to bring order to the vast and complex arena of tannin chemistry this volume of collected papers by leading authorities in this branch of organic chemistry is particularly welcome. A general paper on vegetable tannins (T. White) is followed by a detailed account of estimations of components of vegetable tannin extracts (H. G. C. King and T. White). Descriptive papers on gallotannins and ellagitannins (D. Burton and H. G. Munster), polyphenols of tobacco (W. W. Reid) and paper chromatography of polyphenols of tea (E. A. H. Roberts) together with an account of the work on the constitution of chebulinic acid (O. T. Schmidt) and a summary of aromatic biosynthesis leading to tannins (Hathway) complete the first section. The second part of the symposium is devoted to papers on leuco-anthocyanins (T. Swain and E. C. Bate-Smith; W. E. Hillis), catechins of oak and sweet chestnut (W. Meyer) and dihydroflavonols (J. E. Gowan, E. M. Philbin and T. S. Wheeler). The final paper (W. B. Whalley) consists of an account of the stereochemistry of chromans and related compounds. I feel that another instalment of this symposium would now be most welcome, especially in view of the intense activity in the subject.

A. I. Scott

Handbook of the Petroleum Industry

Edited by G. Sell and H. A. Dossett. Pp. viii+213. (London: George Newnes, Ltd., 1958.) 25*s*. net.

AS the editors are at pains to stress, this is not a text-book on petroleum, but rather a guide to those of the younger generation who contemplate a career in this vast industry. It proves of interest, too, to those oil technologists and others so engaged because of the modern overall picture it presents of the manifold ramifications of the industry. Besides the editors, ten specialists have contributed sections, devoted to geology, history, exploration, drilling and production, world oil resources, distribution, refining, oil shales, tar sands, petrol from coal, oil as fuel, petroleum chemicals, uses and future prospects, careers in the industry and the industry surveyed as a whole. Generally, in the larger oil organizations, the recognized inter-related divisions to-day comprise (*a*) exploration and prospecting, (*b*) drilling and production, (*c*) transport and storage, (*d*) refining and petroleum chemicals, (*e*) research, (*f*) marketing and distribution. Thus a candidate for a career in oil has a wide choice for his or her talents, depending largely, apart from educational attainments and bias, whether overseas travel and work in the more remote parts of the world make fundamental appeal. "Oil is where you find it"; this little handbook tells much about this, as it does about what is done, once found, to make it of universal service to mankind.

THE ROYAL SOCIETY

ANNIVERSARY ADDRESS*

By Sir CYRIL HINSHELWOOD, P.R.S.

AWARD OF MEDALS, 1959

Copley Medal: Sir Macfarlane Burnet, O.M., F.R.S.

BURNET is among the most outstanding workers in the field of the viruses. Since 1925 he has made a long series of contributions to knowledge of the bacteriophages and he has elucidated the phenomenon of lysogenesis. He has shown how study of the range of activity of phages against various bacteria can be used to classify not only the phages but also the bacteria themselves.

He has studied many viruses pathogenic for vertebrates. He has shown how psittacosis is endemic in wild Australian parrots, and has thrown light on the epidemiology of herpes and poliomyelitis. He is well known for his development of several techniques for cultivating viruses in living chick embryos and their application in immunological studies of viruses. He has also made studies on the agglutination of mammalian and avian red blood cells by a number of viruses and virus-products, thereby throwing fresh light on the reactions taking place between viruses and the surfaces of susceptible cells. He found that an enzyme produced by cholera vibrios has an action on blood cells similar to that of influenza viruses.

From 1947 until 1951 Burnet worked on the reaction between influenza viruses and the muco-proteins on cell surfaces, and considered the mechanism whereby a virus can infect a cell. Between 1949 and 1958 he and his collaborators occupied themselves with the theme of genetic recombination between influenza virus particles carrying different, recognizable, marker characters. The possibility of a sort of hybridization between two virus strains was established. Burnet has applied these ideas in an attempt to understand how changes in virulence are brought about.

During the past few years he has turned to the study of fundamental aspects of immunity and has offered in a recent book on the 'clonal selection theory' a novel explanation of antibody production based on the idea of somatic mutation. His ideas about the body's ability to recognize 'self' and 'non-self' to a large extent predicted later findings on immunological tolerance. He applies the new ideas to tentative explanations of auto-immune diseases, of cancer and of ageing.

While so prolific of work and ideas, he has been of outstanding value in the councils of Australian science.

Royal Medal (A): Prof. R. E. Peierls, C.B.E., F.R.S.

Peierls's earliest work was on the theory of the solid state, a field in which he remains a leading authority. He has played an outstanding part in the development of 'fundamental theory', that is, in the formulation of what are believed to be the most basic laws of physics from which all other laws of Nature should ultimately be derivable. The subject-matter of fundamental theory changes over the years. In the late 'twenties and early 'thirties the term was practically synonymous with the relativistic theory of electrons and photons, and even as a very young man Peierls, with Landau, made an important contribution to one of the deepest problems in this subject, that of the limits of observability of physical quantities. When in the mid-thirties Yukawa initiated meson theory and so brought nuclear physics into relation with fundamental theory, Peierls's predominant interest was also in this field. His work with Bethe on the deuteron and that with Kapur on the theory of the compound nucleus are important landmarks of the period.

More recently, Peierls has directed his main, though not exclusive, attention to the general theory of quantal fields and of elementary particles. His best-known single contribution to his rapidly developing topic is a paper on the general derivation of commutation relations for arbitrary field theory, the idea of which has since been built into a great deal of modern work.

For many years Peierls has been a leading exponent of fundamental theory in Great Britain and an outstanding leader in theoretical physics as a whole. In Birmingham he has created a large and extremely active school, and if to-day young British and British-trained theoretical physicists enjoy a great reputation and occupy leading positions, this is largely due to Peierls's work.

Royal Medal (B): Prof. P. B. Medawar, C.B.E., F.R.S.

Medawar's research has been on the biology of senescence, the problems of tissue grafting and the effects of cells upon each other. To these difficult and fundamental subjects he has brought order and clarity, and experimental approaches of great originality.

In some of his earliest work he measured for the first time a property that can be called the growth energy of the chicken's heart, by determining the amount of an inhibitor needed to stop growth. He showed how the rate of decline of growth energy can be interpreted to provide a measure of senescence, and in this and subsequent work he explored with great penetration and caution the various concepts of ageing.

Study of problems of differentiation led him to the hypothesis (with R. E. Billingham) that cells of the adult skin may actively transfer hereditarily transmissible characteristics to their neighbours. In spotted guinea pigs the dark areas may gradually encroach on the light areas, which contain pigment cells lacking the enzymes necessary for melanin formation. The encroachment was shown to be due to

* Delivered at the Royal Society on November 30.

the 'infection' of the light cells by the genes of their neighbours.

Still more fundamental analysis of the problems of tissue inheritance and differentiation followed in Medawar's main work on tissue grafting. A graft from one individual to another will not in general survive (except with identical twins). He showed that this was due to the fact that the graft induces the formation of an immune reaction by the host. The power to react against homografts was, however, found to be prevented from developing if the host animal was injected very young with cells from the donor strain. A mouse, 'tolerant' in this sense, could be made sensitive again if injected with cells from a live mouse of its own strain.

Very recently Medawar and his colleagues have discovered ways of disintegrating cells ultrasonically without destroying their antigenic power. By fractionation it was then shown that this power resides in the nucleus and not the cytoplasm and may be associated with compounds of deoxyribonucleic acid and proteins.

These discoveries have thrown new light on the nature of the differences between individuals and open up important possibilities for their chemical analysis.

Davy Medal : Prof. R. B. Woodward, For.Mem.R.S.

Woodward is by general consent among the leading organic chemists of the world to-day. His most important work has been in the field of natural products, and he has made major contributions to the elucidation of the structures of cantharidine (1941), penicillin (1945), strychnine (1947), sempervirine (1949), santonic acid (1949), patulin (1949), terramycin and aureomycin (1952), cevine (1954), magnamycin (1956), rauvomitine (1956) and gliotoxin (1958).

In the course of these studies he has contributed significantly to our understanding of biogenetic pathways, especially for strychnine and in steroid-triterpene relationships. Characteristic features of his structural work have been the clarity and penetration of his reasoning and the extensive application of modern physical methods. It was by his correlation of ultra-violet spectra with substitution patterns in unsaturated compounds (1941–42) that he made his first important contribution to chemistry. The resultant 'Woodward Rules' have been invaluable, and his own subsequent use of ultra-violet and infra-red methods has been a model to organic chemists.

Woodward's achievements in the field of synthesis are among the most spectacular features of organic chemistry in recent years. His synthetic studies have included work on morphine (1951), and have resulted in the first syntheses of many other important natural products including quinine (1944), sempervirine (1949), patulin (1950), cholesterol and cortisone (1951), strychnine (1954), lysergic acid (1954), lanosterol (1954) and reserpine (1956). Not only are these syntheses notable for their elegance in conception and execution but also they have done much to bring stereospecific reactions into general use in the synthesis of complex molecules and have greatly influenced all recent work in this field.

The scope of his work is further indicated by his demonstration of the aromaticity of ferrocene (1952), his work on reaction mechanisms, especially of the Diels–Alder additions, and most recently his contributions to our understanding of the theory of optical rotatory dispersion.

Hughes Medal: Dr. A. B. Pippard, F.R.S.

Pippard has made outstanding contributions to low-temperature physics, and he must be considered one of the leading experimenters in this field. He has combined first-rate experimental work with a mastery of theory.

Starting with superconductivity, Pippard introduced an important modification to the London theory, a phenomenological description of the electromagnetic properties of a superconductor, which for many years has been an important guiding light in the subject. He was led to this modification by his own experiments on the depths of penetration of currents in superconductors. These were explained by the postulate of long-range wave-functions and thus non-localized effects, the current density at a point depending on the integral of the vector potential over a region (of about 10^{-4} cm.). Later developments in the fundamental theory have tended to confirm the correctness of Pippard's conception.

He also studied the anomalous skin effect in non-superconductors and gave a simple but complete picture of what happens when the mean free path of the conducting electrons is large compared with the skin-depth of penetration of high-frequency currents.

More recently, Pippard has carried out detailed measurements, again at high frequencies, on the surface resistivity of single metal-crystals, analysing the variations which occur in different directions related to the crystal axes. In his work on copper, he has shown how such measurements can lead to a plot of the 'Fermi surface' of the metal. By this is meant that a contour can be plotted of the way in which the positions of the valence electrons of highest energy are woven into the crystal-lattice structure. This adds to our knowledge from X-ray crystallography of the lattice structure of the nuclei in a crystal, fundamental information about the geometrical pattern of the electrons.

Pippard's work is characterized by deep originality, coupled with experimental ingenuity and a profound theoretical insight into his subject.

THE INTERNAL AND THE EXTERNAL WORLDS

There has been no slackening in the flow of significant discoveries from the world of science. The most spectacular event of the recent past has been the transmission from the Russian *Lunik* of photographs showing the remote face of the Moon, an achievement acclaimed equally by the layman and the man of science. In a more recondite way interest has been aroused by an electrostatic theory of cosmological expansion, and in contrast with these escapes, in action or thought, beyond the bounds of the Earth, plans have been made for unprecedentedly deep drilling into its interior. Syntheses such as that of coenzyme A and of 6-amino penicillanic acid have further strengthened the alliance of organic chemistry and biology, and the production of specific forms of deoxyribonucleic acid outside a living cell but primed by the presence of existing deoxyribonucleic acid has been another step towards the understanding of the material basis of life.

Any of these things, and indeed many others, might well form themes for a presidential discourse, but with a special end in view I shall single out for mention one or two isolated and indeed rather specialized samples. These are chosen because they not only involve fascinating discoveries but also because they lead us to ponder once again on deep questions which should not be ignored by the contemporary man of science. They relate, in fact, to the concomitance of the inner world of conscious experience and the outer world of Nature. This extraordinary problem is always with us: but the unexpectedness of the new observations, though it does not really disclose new kinds of mystery, does reawaken us to awareness of something, the strangeness of which familiarity tends to dull.

The social life of insects cannot but fill the onlooker with wonder, and arouse speculation about the conscious elements which may lie behind the behaviour manifest to an observer. Nobody intimately acquainted with individuals among the higher animals can seriously doubt that they possess some inner life, perhaps not entirely unlike our own. But that of the insects is by our standards likely to be very peculiar indeed: yet there is in all probability an intelligible pattern to it. The Rothamsted work on the 'queen substance', transmitted from the queen bee to the other members of the hive, shows how this chemical compound regulates the whole reproductive process of the bee community and dominates the organization of their lives.

The inward concomitants of the observed behaviour we can scarcely imagine, and indeed the control of highly complex individual and communal actions by a chemical substance could easily suggest a purely behaviourist view of living creatures, and lead to the denial of the internal world altogether. This I believe to be philosophically wrong.

Another recent research provokes us to think about the matter, as it were in the reverse order. We are familiar enough with human colour vision and can scarcely doubt the existence of this as a personal experience. The external conditioning of it has recently been stated by Land to differ very surprisingly from what had been supposed, and to be of a quite extraordinary character. The essential experiment is made in the following way. A coloured scene is photographed by means of a split-image camera simultaneously through two filters, one of which cuts off the upper half of the visible spectrum and the other the lower half. Two black-and-white positive transparencies are thus prepared. These are projected separately each with a different monochromatic light, for example, two yellows differing in wavelength by a few hundred angströms, and the images are recombined. The resultant produces in the observer the whole gamut of colour sensations. Thus information can be supplied to the brain so that, as Land puts it, "the eye functions to see full colour in a universe involving only a narrow wavelength band".

The question of the relation of the internal to the external world is again brought into sharp relief by these remarkable findings.

The topics I have mentioned can, of course, be treated without specific reference to these problems, but they lose a great deal of their interest if the implications about consciousness are ignored or denied. There is indeed one group of researches, carried further during the past year, in which these implications cannot possibly be evaded, namely, those in which physiological stimulation of the conscious brain is correlated not only with the behaviour of animals but also with the psychological experiences of a human subject.

The central problem of mind and matter is not always thought worthy of their attention by men of science, yet it was given profound consideration several decades ago by Sir Charles Sherrington, and we are vividly reminded of it in the most recent book of one of our senior Fellows, Lord Russell. At a moment, too, when Darwin is again very much in our minds, there are certain aspects of the whole matter about which it is highly important to think clearly. The question raises a still more general issue which I should first like to consider. There are few worse condemnations of a theory than to label it metaphysical, a term suggesting the attempt to arrive at truth by speculation divorced from experiment or to interpret reality by statements empty of scientific sense. Little enough enlightenment is afforded by such an assertion as that the orbit of a heavenly body has a given geometric form because this constitutes the perfect figure. Scientific theories are properly expected to account for many things in terms of few, to predict new phenomena or to yield verifiable, quantitative results. Yet too austere an application of this principle can segregate the world of science from the general world of intelligent appreciation, an unfortunate event at a moment when there is growing realization of the interest of science to the community at large.

Most men of science would disclaim any great concern with philosophy and especially with metaphysics. But what is metaphysics? In the last resort it is the search for a statement subsuming the ultimate nature of all reality. Science has more modest hopes, and modern physics led the way to a considerable renunciation in this respect which has had a profound influence on philosophy itself.

Yet in science the total neglect of philosophical aspects may be unfortunate. It can lead, on one hand, to statements which are sensational but unsound, and, on the other, to an unhealthy indifference to the relationship of science to existence in general; and in any event, even though science may seem to decline metaphysical statements, it not only throws out challenges to philosophy but also is frequently itself confronted with problems inescapably philosophical in nature.

Scientific theories, though less ambitious than the systems of the older philosophers, and though claiming neither universality nor finality, do aim to be comprehensive and do hope to stand the test of time as long as possible. Moreover, we do judge them by a criterion in which it would be hard to disclaim every metaphysical tinge, namely, by the degree of mental and æsthetic satisfaction they afford. However much we may protest, utility is not enough. There is, undeniably, in the law of gravitation some quality distinguishing it from a set of empirical formulæ, however convenient. Men of science do, unconsciously at least, place hypotheses about Nature in a sort of hierarchy of esteem, where the conservation of energy and the second law of thermodynamics rank high and mere working rules rank low.

The difficulty of complete aloofness from philosophy is illustrated by parts of physics. The theory of relativity dominates cosmology and defines the relationship between matter and energy. The quantum laws and the Pauli principle provide the foundations of chemistry. Yet these powerful and

productive generalizations involve distinctly metaphysical elements. Although it seems to have been relatively late in the development of thought that a clear distinction between subject and object, between the external world and the observer was recognized at all, for most men of science there was not much question about it at the beginning of the present century. Einstein, however, asserted the meaningless character of space and time abstracted from operations with measuring instruments and clocks and thus established the observer as an indispensable participant, whose status was increased by the 'uncertainty principle'.

According to the Pauli principle, observability becomes identifiable with the very possibility of existence. Thus the foundations of inanimate Nature can scarcely be discussed without recognition of the inadequacy of earlier spatio-temporal descriptions of reality, and without admission that subject and object had been in some respects too crudely separated.

The fundamental laws of quantum physics can be summarized in the wave equation and in a set of rules for its operation. Some people, it is true, say that they are content to accept these without deeper inquiry. But in fact every one, whether he admits it or not, seeks something which he calls understanding. The professed empiricism about wave mechanics is half-hearted, and more often than not the abstract mathematical formulation is given a metaphorical translation in which symbol and reality become very much confused. 'Waves of probability' are created, electrons become 'clouds', potential barriers are 'tunnelled through'.

When sound metaphysics goes, the seven devils of bad metaphysics soon enter to fill the gap and give an illusion of clarity. Metaphors which may be helpful in illustration easily take control when the abstractness of the mathematical formulation becomes too austere, a fact which bears witness to the insistent demand of the mind for satisfaction by something beyond utility.

Incidentally it may be remarked that if the more serious and responsible men of science remain aloof from the task of relating their subject to its philosophical background, the less serious and responsible will do it for them, and will startle the uninitiated with fantastic paradoxes which may impress the layman, but do science real harm. The discovered facts of Nature are mysterious and impressive enough in their own right. That light seems to recede from relatively moving observers at the same rate: that the universe may look much the same from anywhere: that measured mass may vanish into energy of radiation: that states of energy form a discontinuous series: that indistinguishable particles cannot coexist: that in the world of small enough dimensions particles and waves lose the significance suggested by macroscopic analogies: none of these needs dressing in sensational form to make its impact on the thoughtful mind.

Nor, on the other hand, are they merely to be made the basis of formulæ for the sole purpose of computations. In the last resort what men of science search for is the vision of Nature, and this must be expressed and communicated in those terms which provide the nearest approach to real understanding.

If this is true of the physical sciences, it is equally so of biology. One of the transitions from physics to biology is illuminated by a remark of Heisenberg: "The mathematical formulæ indeed no longer portray nature but rather our knowledge of nature".

The question, then, of the relation of the internal and the external worlds cannot and should not be ignored by men of science.

It is surprising that biological discussions often under-estimate human consciousness as a fundamental experimental datum. In science we attach no value to unverifiable deductions, or to empty qualitative statements, but nobody defends the neglect of experimental data. Among these we cannot validly disregard those of our own consciousness except by a deliberate abstraction for which we must assume responsibility, and which we should not forget having made.

It is tempting to ignore observational facts when they seem intractable, since we rightly abstain from the attempt to arrive at the truth by speculation. But in this we are not always consistent, and indeed very important philosophical conclusions are sometimes propounded by men of science. Moral and æsthetic qualities in man, for example, are not infrequently said to be explained by the operation of natural selection. Again, it is sometimes asserted that suitable combinations of machines could, in principle, produce results indistinguishable by any operational test from animal or even human behaviour.

The mind–matter relation is quite often dismissed as non-existent or meaningless. But moral qualities are not explained by natural selection, human behaviour is not machine-like and the mind–matter relation cannot be ignored in an intelligent consideration of existence.

The approach to the problem of consciousness must admittedly be egocentric. I have to begin logically with the, to me, indisputable fact that I have a wide range of conscious experiences (which are indeed more important to me than anything else). The significance of this fact can be questioned in two ways: first, by the objection that so far as any operational test goes other people may well be behaving simply as machines, and secondly, by the assertion that my consciousness is a more or less irrelevant concomitant of behaviouristic reactions.

I am, it is true, not certain that other people have consciousness. Neither indeed am I certain that the atomic nucleus exists. But I regard its existence as in the highest degree probable. I regard consciousness in other people as equally probable and for similar reasons. The atomic nucleus is not directly observed. It is inferred by elaborate reasoning from many complex experiments, any one of which could probably be given alternative interpretations. What carries conviction is the fact that a coherent body of doctrine emerges from large numbers of varied tests.

We are all the time trying experiments on our relations with other people, informing, asking, ordering, obeying, resisting, with varying emotions which are correlated with our actions. Human evolution has developed an elaborate and sensitive communication system, brought into play in innumerable ways. The hypothesis that other people have an interior life not unlike my own enables me to register correspondences at point after point in so intricate a way that I accept the hypothesis, if not as absolute truth, then as something nearly as good. And with most of the basic conclusions of science I am in no position to demand more.

Thus the content of consciousness must rank as something directly experienced by everyone, and

therefore as providing observational data. This conclusion is not changed in the least by the consideration that the development of communication between individuals confers a biological advantage and is favoured by natural selection in so far as mutual assistance and the transmission of acquired knowledge can assist survival. The communication mechanisms exist and one of their uses is to enable us elaborately to test the coherence of predictions from the hypothesis that conscious life is on the whole much the same for different people. People like poets and novelists describe this inner life: their writings serve little enough biological purpose, but they are (sometimes at least) intelligible to the rest of us, and evoke memories of our own experiences, which check as significantly as those coincidences of calculations and dial readings on which we base our views about the physical world.

Nor can the observational data of consciousness be relegated on the ground that they are on the whole qualitative rather than quantitative. Science has to come to terms with qualities, and indeed Weisskopf has pointed out that the quantum theory itself introduced fresh qualitative distinctions into a region where classical physics expected only quantitative differences.

The conscious experience is sometimes set aside as supernumerary and irrelevant, on the ground that a machine can perform actions indistinguishable from those of a man (or with greater technical perfection could do so). The answer that machines like the so-called electronic brains work only to a 'programme' brings the rejoinder that a set of machines could itself be programmed by another machine. But the master machine would itself need a programme. This would have to be provided by another machine, and so on in an infinite regress, there being no way in which the sequence could be closed to a self-contained cycle.

The contention that a human being is a system of self-programming mechanisms seems to depend upon the error of neglecting relevant observational facts, those of consciousness belonging to precisely this category. In its higher functions the human brain is programmed, not by other mechanisms, but by æsthetic and moral elements which somehow have their seat in the consciousness, by elements, that is to say, which belong to the half of reality concerned with the observer rather than the observed. Of the concomitance of consciousness with brain processes there is no doubt, but concomitance does not mean identity. Changes in the electron-levels of an atom are associated with the emission of light quanta, but a photon is not a difference of quantum states. Nor in a concomitance of A and B is it justifiable to assert that A is the cause of B without admitting the possibility of a reciprocal relation. In particular, if we assert that physical happenings in the brain can affect consciousness, we must admit in principle that physical happenings in the brain may be consciously caused.

But it is an abstraction to speak of either category in isolation. For many purposes the two may be separated as a legitimate approximation, but this does not mean that they can be regarded as independent.

In parts of biology questions of consciousness are not of direct importance but cannot in general be set aside. Behaviourism might give a good approximation in describing the actions of intercommunicating individuals in a group, but it ignores the experimental datum, known to each individual about himself, and, by the coherence argument, about others, that in their conscious minds what men individually think or feel is of far more importance to them than any mechanical part which they may play in the biological community.

There is, of course, a clear enough meaning in the statement that consciousness and moral and æsthetic feelings are developed by evolution and natural selection. The power of conscious reasoning increases the chance of individual survival: social instincts favour the survival of the community, and are related to moral feelings. Æsthetics enter deeply into intellectual activity. There need be no difficulty in agreeing that these things have selective advantage. But the recognition that something present will be selected gives no clue whatsoever to its actual nature or genesis. For the purposes of natural selection a purely unconscious reaction of the brain and nervous system would have sufficed. Beneficial reactions indeed need have no conscious counterpart, as is widely evident in human physiology. But behaviouristic descriptions in general are models and abstractions ignoring data which, so far from being trivial and irrelevant, are the only things which make us *inter alia* concern ourselves with science at all. In any event, if free will, moral feelings and other conscious elements are illusions superposed on a behaviouristic background, wherein can lie their selective advantage?

There is at present no obvious answer to the question of what kind of advance can possibly be hoped for in the problem of psycho-physical concomitance. This, however, is no reason for giving up thought which at least helps to avoid the kind of errors so easily made both about physics and about biology when the problem is ignored.

It would, moreover, be a mistaken policy for men of science to withdraw from the inquiry. Lucretius might well have been asked how any real knowledge of his primordial particles could ever be obtained and would have been unable to answer. Before Galileo and Newton there were no appropriate concepts in terms of which to understand the flight of a projectile. At one time the rusting of iron must have seemed a process *sui generis*. The discoveries of science and the evolution of its grammar go hand in hand. Negative predictions are discredited by the famous pronouncements of Comte: "Toute recherche qui n'est point finalement réductible à de simples observations visuelles nous est donc nécessairement interdite au sujet des astres. . . . Nous concevons la possibilité de déterminer leurs formes, leur distances, leurs grandeurs, et leurs mouvements; tandis que nous ne saurions jamais étudier par aucun moyen leur composition chimique".

In the earliest times the description in rational terms of physical Nature was not so much an unsolved problem as one which people did not even conceive.

Human knowledge will not be in a satisfactory state until the dichotomy of the internal and the external is somehow removed.

In one way the man of science, because he starts by demanding least of an explanation, is the most likely to succeed in finding it. The question might be asked: What happens when an atom emits light? The more naïvely this is considered the less is an answer possible. But the laws governing various correlations, change of atomic state with quantum emission, or quantum with field have been formulated in terms of concepts appropriate to the problem, and

they yield satisfaction not only by making possible quantitative predictions but also by an inherent elegance. Eddington once discussed the way in which the terms of physics link together in endless cycles and pointed out that in one way Newtonian mechanics constitutes a vast tautology. This is often true in so far as physical theories create, as it were from nothing, a series of definitions and concepts by the aid of which things previously intractable are brought together into order and intelligibility.

In the light of this analogy, the further exploration of the concomitance of the internal and external worlds appears less permanently hopeless. The right grammar and language need to be evolved. In the process the present bounds of science may expand, but they have never been established by a firm decree. Chemistry and physics have united with biology and penetrated regions once thought inaccessible. Physiology is already allied with psychology and together they may search in strange and hidden places. There will no doubt be difficulties of which Freud's struggle for acceptance provides a warning example, but it may be recalled that chemistry and physics themselves arose from a welter of charlatanry, magic and surmise as the correct language in which to describe their phenomena grew up. There are so-called para-scientific aspects of biology and psychology, which to-day show all too often the discouraging characteristics of the worst days of alchemy. Yet the history of chemistry warns against a final condemnation.

In the meantime those numerous studies are to be welcomed which, without solving the central problem, keep it constantly alive: observations on individual and communal behaviour in animals, on perception and illusion, investigation of the processes of learning and memory, construction of machines which imitate the mental functions, search into the phenomena of sleep. Above all, there lies hope that sooner or later to the eye of genius some quite ordinary phenomena will reveal, like the fall of Newton's apple, an undreamt of but thenceforth obvious significance.

THE SHAPE OF PROTEIN MOLECULES SUGGESTED BY ELECTRON MICROSCOPY

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THE weights of most of the important protein molecules are now known with a fair degree of certainty. Apart, however, from some of the fibrous proteins such as collagen, speculation about their shapes and sizes is less surely based. The weight of a protein molecule when multiplied by the readily determined value for its partial specific volume gives the volume of the anhydrous molecule. Therefore, if protein molecules were compact spheres, their diameters would be known, and values calculated in this way from available data[1] are listed in Table 1 for various proteins. But a diameter can also be calculated[1], again on the assumption of a spherical molecule, from the hydrodynamic behaviour of the protein, in particular from its rate of diffusion and sedimentation in the centrifuge and the viscosity of its solution. In almost every case this diameter comes out to be significantly larger than that indicated by the molecular weight, the ratio of the two values giving the frictional ratio for the molecule. There are three possible explanations for the discrepancy, that is, for frictional ratios greater than unity: (1) the molecules may carry water either internally or as a surface layer, and thus behave in solution as larger objects than the calculated compact anhydrous size; (2) the molecules may be elongated rather than spherical; (3) the hydrodynamic formulæ involved in the calculations may not be strictly applicable to protein molecules.

The third explanation is not always considered, but in fact there is no compelling evidence that molecules behave like smooth, rigid bodies, as theoretically required; nor indeed any direct evidence that the formulæ used are valid for objects as small as a molecule. The two other possible explanations are usually both taken into account and lead in the following way to a picture of the molecular shape. The extent to which proteins are hydrated is not easily measured, but 0·3 gm. water bound per gm. protein is thought in most cases to be a good estimate. Assuming some such figure, one can next calculate from theory[2] the ratio of the length to the width of the molecule that would then account for its behaviour. Table 1 shows the results of these calculations. The molecules are assumed to be ellipsoids—either prolate (cigar-shaped) or oblate (disk-shaped)—but provided the structure is rigid, the length-to-width ratios are not critically dependent on the exact model. Except for ferritin, all the proteins listed come out to be distinctly elongated, and it is as long prolate ellipsoids that these protein molecules are usually visualized.

The electron microscope, with its limit at a resolution of 15 A., ought now to be capable of confirming these theoretical deductions by showing the shape of the anhydrous molecules directly on a photographic plate. That it does not do so is due to the low electron-scattering power of proteins, and in practice no untreated molecule with a molecular weight less than about 2 million can be detected at all. However, the contrast is greatly increased by metal shadowing and in this way Hall has obtained some fine pictures, for example, of catalase[3] and fibrinogen[4]. But such pictures require exceptional technical skill, and as usually employed the metal coating is too grainy to give a reliable picture. In addition, the structure of the supporting film is inevitably brought out and can cause confusion. But even allowing for these difficulties, it is curious that in every attempt so far published the electron microscope has failed to show the expected elongated forms of the protein molecules.

A method that shows very fine detail on the surface of virus particles has recently been developed by Brenner and Horne[5,6] and which they have termed

Table 1

Protein	Molecular weight	Volume of compact anhydrous molecule (A.^{3} × 10^{-4})	Diameter of molecule if a compact sphere (A.)	Ratio length/width of molecule to account for behaviour if 30 per cent hydrated	Hydration required to account for behaviour if molecules are spheres (per cent)
Glutamate dehydrogenase	$1{\cdot}0 \times 10^6$	1·25	134	3·5	95
Thyroglobulin	$6{\cdot}3 \times 10^5$	0·76	113	4·2	120
Urease	$4{\cdot}8 \times 10^5$	0·59	104	3·2	85
Ferritin	$4{\cdot}7 \times 10^{5*}$	0·59*	110	1·0	30
Legumin	$3{\cdot}8 \times 10^5$	0·46	96	7·0	230
Catalase	$2{\cdot}5 \times 10^5$	0·31	84	3·4	90
γ-Globulin	$1{\cdot}6 \times 10^5$	0·20	73	5·9	190
Serum albumin	$6{\cdot}6 \times 10^4$	0·08	54	3·4	95

* Figures refer to the protein component of ferritin which in addition contains 20 per cent iron.

'negative staining'. Using spray droplets, they partly embed the virus in a layer of neutralized phosphotungstic acid and the relatively transparent objects appear with great contrast against a nearly opaque and structureless background. This is the reverse of the usual appearance and 'negative contrast' is perhaps a better description of the effect achieved. It seemed that a similar method might also be useful for showing up the larger protein molecules. For this application the original procedure was modified as follows. The protein at concentrations of between 0·1 and 1·0 mgm. per ml. was made up in 0·02 *M* phosphate buffer *p*H 7·8 and an equal volume of 1 per cent sodium tungstate added. The technique depends essentially on avoiding any interaction between the tungstate and the protein, and for this reason the *p*H was maintained well above the isoelectric point. It was found helpful for the electron microscopy to add polystyrene latex spheres of 0·2μ diameter[7], which can be seen easily on the screen in negative contrast (that is, bright on a dark background), if the preparation has been satisfactory. The protein solution is sprayed on to the specimen supports exactly as described by Williams and Backus[8]. When the electron micrographs of the preparation are examined, the protein molecules appear as clear points in 5–20μ droplets of dark structureless material. The procedure is rapid and some of the risk of distortion by surface forces is avoided since the drops set rather than dry. The molecules should be well preserved, and in fact simple tests have shown that proteins dried in the droplets and then re-wetted are still largely undenatured (for example, enzymes remain active).

Experiments with purified preparations of the larger proteins soon showed that while some gave pictures of high contrast, demonstrating convincingly the power of this new technique, others were almost equally disappointing. This was not related to molecular size, for in fact some of the smaller molecules appeared with the greatest clarity. The difference may possibly lie between proteins that are internally hydrated and those that are not. The tungstate can penetrate very small holes (this sort of technique was first used to demonstrate the 15 A. hole along the axis of the tobacco mosaic virus rod[9]) and any molecule having an open sponge-like structure may fill with tungstate and be almost obscured. Although preventing good pictures of some interesting proteins from being obtained, this penetration may indicate which molecules have extensive internal hydration.

The proteins listed in Table 1 were all examined by this negative contrast technique, and some of the pictures obtained are shown in Figs. 1–6. Points particularly noted about each are now discussed.

L-*Glutamate Dehydrogenase* (*Glutamic Acid Dehydrogenase*). The unusual behaviour of this protein in the ultracentrifuge[10] has suggested that the molecule can dissociate reversibly on dilution into smaller units. Similar dissociation has also been observed in the presence of substrate[11], while in the presence of coenzymes re-association occurs[12]. The molecular weight of the undissociated molecule has been measured as 1,000,000 (ref. 10), and it is suggested[11] that this represents a tetramer that can split into a dimer and then a monomer. Our electron micrographs lend support to these ideas. The objects seen varied in size, depending on the concentration at which the protein was sprayed. At suitable dilutions (about 0·5 mgm. per ml.), three sizes of roughly spherical objects could be seen (Fig. 1) measuring 125 A., 90 A. and 75 A. The cubes of these numbers (which give a measure of volume) are almost exactly in the ratio 4 : 2 : 1. Smaller debris was also usually present. The largest objects appeared slightly smaller than the size of 134 A. calculated for a molecule of the molecular weight reported, but the difference might not be significant. While these electron micrographs certainly support the picture of the molecule as a tetramer, they do not suggest a simple aggregate of 4 units. On dissociation, the molecule appears as if it has broken into two or four equal pieces which have re-formed into round irregular shapes. Thus although dissociation is said to be accompanied by a fall in axial ratio[11], there is no obvious change in shape to account for this.

Thyroglobulin. Despite its large molecular weight, the molecules of this protein were almost indetectable by the negative-contrast technique. Vague round objects measuring about 140 A. across could just be made out, and objects of similar size were seen in metal-shadowed preparations. It may be that this molecule has an open structure that is easily penetrated by the sodium tungstate and occupies a volume roughly twice that calculated for a compact molecule of this weight.

Urease. The molecules were similarly scarcely detectable. A diameter of about 130 A. was suggested, again giving a volume almost twice that of a similar compact molecule.

Ferritin. The structure of this molecule deduced from X-ray scattering has been described recently[13] and the findings correlated with previous work. The molecule contains 20 per cent iron (as ferric hydroxide) at its centre, this iron being free to move in and out

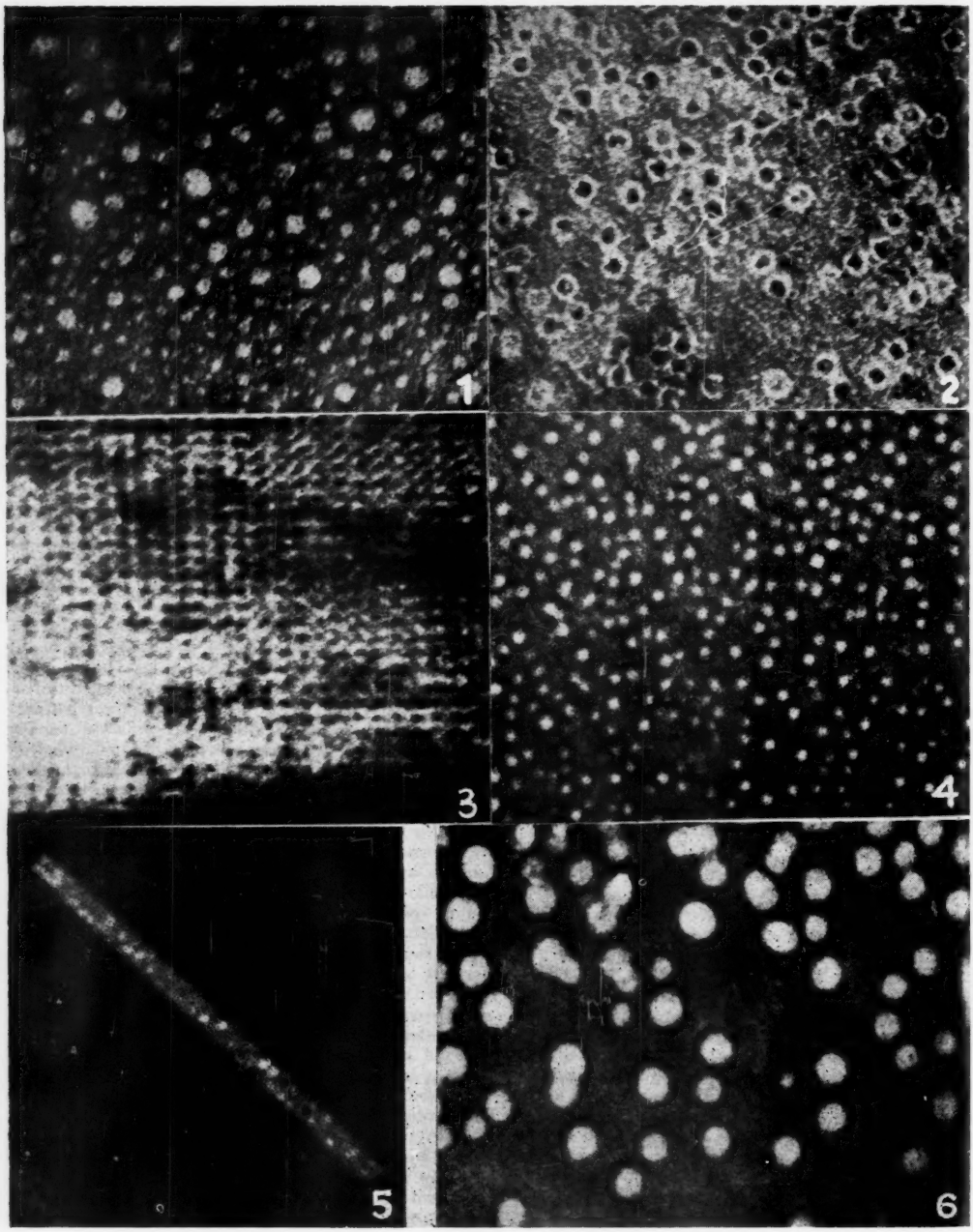

Fig. 1. Molecules of L-glutamate dehydrogenase. × 400,000

Fig. 2. Molecules of ferritin. × 400,000

Fig. 3. Molecules of catalase in a small crystal. × 400,000

Fig. 4. Molecules of γ-globulin. The centre to centre spacing of molecules that touch is 70 A., but the extreme edge of the molecules is not clearly shown on the print. × 400,000

Fig. 5. Tobacco mosaic virus rod with molecules of γ-globulin antibody on its surface. × 250,000

Fig. 6. Molecules of macroglobulin. × 400,000

without disarranging the protein shell. The form of the iron core has already been studied with the electron microscope[14] and its varying appearance interpreted as differing aspects of four closely spaced micelles. The negative contrast pictures (Fig. 2), while still clearly showing the central masses of iron, give a better picture of the outline of the surrounding protein. Some of the molecules can be seen to be partly or completely deficient of iron. The protein shell is about 25 A. thick and the diameter of the molecules measures 110 A.

Legumin. A sample of this plant globulin was kindly supplied by Dr. P. Johnson (Department of Colloid Science, Cambridge) and the full physical data and findings will be presented elsewhere. The negative-contrast technique showed the molecule as a short cylinder 85 A. in diameter and 85 A. high. A hole 15 A. wide was seen to run along the axis from one end of the molecule to the other. The volume of such a solid corresponds closely with that calculated from the molecular weight, but the electron microscope again completely fails to show the asymmetrical shape required to fit the hydrodynamic data.

Catalase. The molecules of catalase appeared as slightly elongated rings or as two parallel lines, suggesting end-on and side-on aspects of a hollow approximately cylindrical shape. The identification of such objects as catalase molecules was confirmed by obtaining pictures of the surface of small crystals of the protein (Fig. 3). The same ring-like forms can be seen in orderly array, the molecules measuring 60 A. across their short axis and 85 A. across the long one. The molecules in the crystal appear to interlock almost completely, not just to touch ; thus the width is very little greater where two rings meet in the crystal than it is in a single ring in isolation. This may suggest that the molecule has some sort of helical form that allows the surfaces to interlock. The height of the molecules cannot be deduced from the electron micrographs of the crystal, but measurements of isolated molecules seen on their sides suggest a height of 70 A. From metal-shadowed pictures of catalase crystals, Hall[3] has deduced a height of about 60 A. The thickness of the ring when the molecule is seen end-on is about 20 A. and this figure with a height of 70 A. fits well with the molecular weight of 250,000. The suggested shape is also consistent with Hall's pictures of catalase molecules[3], but the granulation in the metal shadowing partly masks the appearance of rings, and as a result he interpreted the electron micrographs differently.

γ-Globulin. The molecule is traditionally pictured as cigar-shaped with a length-to-width ratio of about 5 : 1. The negative-contrast technique shows a perfectly round molecule of diameter 70 A. (Fig. 4). Similar molecules were seen attached to viruses after treatment with specific antiserum. Fig. 5 shows a tobacco mosaic virus rod after such treatment and a number of γ-globulin molecules (antibodies) can be made out on its surface.

Serum Albumin. Repeated attempts to show the molecule of albumin in the same way have failed. All that has appeared are vague round forms 70 A. in diameter and objects too small to be the intact molecules measuring 30 A. A possible suggestion is again that this molecule has an open structure largely penetrated by the sodium tungstate.

Macroglobulin. A sample of globulins from a pathological serum was examined. In the ultracentrifuge this had shown, in addition to the usual γ-globulin peak, other peaks at 16·2 *S*, 22·5 *S* and 29·6 *S*. The electron micrographs of this material (Fig. 6) showed various sizes of spherical molecule with diameters close to 70 A. (normal γ-globulin), 100 A., 125 A. and 150 A.

This preliminary survey of protein molecules with the electron microscope has clearly shown two points. The first is that the negative-contrast technique is a valuable method for showing the shape of many molecules, and the possibility of studying molecular interactions in the same way is opened up. Secondly, the question that has frequently been raised of late as to the significance of the frictional ratios calculated for molecules is now even more in dispute. In the case of ferritin, the values obtained[15] for the sedimentation and diffusion constant fit well with the observed nearly spherical shape (the axial ratio calculated was originally given as 3·4, but this seems to be an error). The nearly symmetrical but not spherical shapes found for catalase and legumin would have an undetermined effect on the frictional ratio, but could scarcely give values as high as those actually found. The loose open structures proposed for thyroglobulin, urease and albumin might, however, have high frictional ratios. But in the case of γ-globulin a perfectly spherical form behaves as an ellipsoid five times as long as it is wide ; to account for this a hydration of almost 200 per cent would have to be proposed and this will generally be considered an impossibly high figure. If one is not to conclude that frictional ratios bear little relation to shape and merely indicate the degree to which classical hydrodynamics is inapplicable to the molecules, only one alternative seems to remain. It is possible, as Hall has suggested for fibrinogen[4], that the symmetrical objects seen with the electron microscope are only units of the molecule and are joined in pairs or trios by undetectable links. Thus γ-globulin could possibly be a dumb-bell-shaped molecule with its spherical ends linked by perhaps a single protein chain that would remain unseen if less than 10 A. wide. Study has, however, so far quite failed to produce any evidence in favour of such pairing of units. With all the proteins studied here, the electron microscope in fact suggests various almost symmetrical but not always spherical shapes.

I am grateful to various colleagues, Dr. A. S. McFarlane, Dr. J. R. Tata and Dr. A. C. Allison, for samples of the purified proteins used in this work, to Dr. P. A. Charlwood for ultra-centrifuge data on the macroglobulin and to Miss B. Cooling for help with the electron microscopy.

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OBITUARY

Prof. C. T. R. Wilson, C.H., F.R.S.

At the time of his death, and in relation to date of election, Charles Thomson Rees Wilson was senior Fellow of the Royal Society by four years. He was elected to the fellowship in 1900, and, indeed, of those elected in the decade 1900–10, only three survive him. He received the Hughes Medal in 1911, a Royal Medal in 1922, and the Copley Medal in 1935. Eight years previously he had shared the Nobel Prize for Physics with A. H. Compton. This bare recital of fact tends to obscure the man in the record of history: it closes in the year which followed his retirement from the Jacksonian professorship at Cambridge, and his return to Edinburgh—and Scotland. It obscures C. T. R. the man, who endured, until the end, bright-eyed, nervously alert, long of stride, young of heart, serene through a quarter of a century of conflict and change which passed him by.

His years of retirement were not empty. Soon after his eightieth birthday, he moved from Edinburgh to the village of Carlops, close to his birthplace at the farmhouse of Crosshouse, at Glencorse; but regularly thereafter he made the journey to the city by bus so that he might lunch with friends, physicists and others, many of half his age or younger, once a week in a crowded restaurant. Scientifically, too, his retirement was fruitful: some four or five years ago he began again to write the long-promised account of his theory of thundercloud electricity, and finally, on April 27, 1956, the manuscript was lodged with the Royal Society. It was published in the *Proceedings* in August of the same year: twenty pages of closely reasoned statement—emphatically a work of the present, not of the past. Nor was he homebound: he attended a dinner in his old college at the time of his eightieth birthday and, when the reactor *Dido* was inaugurated at Harwell in November 1956, he was among the guests. It was my privilege to be his travelling companion on that November day. Rising about 5.30 a.m., he was in Edinburgh soon after seven; we made the return journey to London Airport by B.E.A. *Viscount*; it was nearly 11 p.m. before he was home again. In the meantime, everything that there was to be seen he had seen; he had climbed all the ladders and looked into all the peepholes; there had been a diffusion cloud-chamber working, and he had seen that too. He was delighted. Only one discontent clouded his joy. Travel by civilian air-liner was not overmuch to his liking. His preference was for the rigours and the excitement of the ancient *Anson* of the weather flight, in which he was flying as a student whenever occasion offered, an honorary member of the meteorology class of the University of Edinburgh. Such flights were a recurring delight of his last years, taking him to the north-west, over the outer isles, in fine weather and foul.

C. T. R. lived to become a legend: for me he was already legendary nearly forty years ago. The headmaster of my Yorkshire grammar school had been head of the science side at Bradford in the 1890's, and there, for a brief spell, C. T. R. had endeavoured to instruct the young, soon after he had graduated at Cambridge in 1892. In later years, when he had retired from Bridlington, Arthur Thornton reminded the authorities of his former school of a forgotten episode of its history, how for a little while it had numbered among its staff a future Nobel prizeman—and had not known his worth.

Wilson's father died when he was four years old. Thereafter his mother moved to Manchester, and C. T. R., intending to become a doctor, entered Owens College to study biology. Balfour Stewart (an Edinburgh man) was professor of physics at the time. A dozen years previously, or thereby, J. J. Thomson had passed through his class on his way to Cambridge. C. T. R. went on to Cambridge, also: to Sidney Sussex College, with an entrance scholarship. Not until then did his interest veer to the physical sciences; but once the decision had been taken, that he should abandon medicine, there was no turning back. By the beginning of 1895 he had already started on what was to prove his major work. It was an unlikely beginning for a fundamental research, yet, after thirty years, it was to fill the physical laboratories of the world with an apparatus which, for a generation, did more to speed the progress of experimental physics than any other agency.

In 1895, Wilson was merely attempting "to imitate in the laboratory" the 'glories' which he had seen on the summit of Ben Nevis in the late summer of the previous year. But he had a sharp eye, and a keen mind, and he had been working for no more than a few months before his interest became engaged in the few drops which reappeared time after time continually, as often as he chose to expand a volume of moist, dust-free, air by more than a 5 : 4 ratio. He seized the significant conclusion that in such circumstances condensation nuclei must be being produced continuously throughout the sample of air. This observation could not have been made at a more opportune time—or in a more suitable place. In December 1895 news of Röntgen's discovery startled the world; towards the end of the following month, in Cambridge, Thomson and McClelland discovered the conductivity imparted to air by the passage of X-rays. Old knowledge concerning the slight 'residual' conductivity of the atmosphere now began to fit into place, and it was with 'delight', but scarcely, one imagines, with surprise, that Wilson found his 'rain-like' condensation enormously increased when he exposed his primitive cloud chamber to the new rays. This was in February 1896. The hypothesis that the conductivity was due to gaseous ionization was firmly established by Thomson and Rutherford during the summer of that year. Thereafter no one doubted that gaseous ions could be fixed in this manner—by condensing water vapour on them, through the process of adiabatic expansion.

Characteristically, Wilson did not himself become involved, at that time, in the excitement of the new investigations into the other properties of X-rays or of the Becquerel radiation. Instead, he carried out a full investigation of the details of the condensation phenomenon and, with simple apparatus, but sure insight, a thorough study of the residual ionization in air and other gases. Then, in the early years of the century, he turned to different problems, and an increasing load of advanced teaching came to

occupy his time. In 1910 a second phase of interest in the cloud apparatus followed, and early in 1911, for the first time, the tracks of individual α-particles and electrons were seen and photographed. The paths of the α-particles were just as W. H. Bragg had drawn them, five years previously, and the tracks of the electrons "little wisps and threads of clouds". This achievement was widely—and justly—acclaimed but, strangely, the literature of the period provides little evidence that others elsewhere sought to emulate Wilson's success.

The situation was entirely different after the third phase of Wilson's own interest. By 1923 he had finally brought to perfection this most remarkable method of experiment. The photographs of electron tracks with which he illustrated two classic papers published in that year were of such quality—and beauty—that the cloud chamber could no longer be dismissed as an amusing toy. All over the civilized world workers built themselves the necessary equipment. In Cambridge, Blackett and Kapitza; in Paris, Irène Curie and Auger; in Berlin, Bothe and Meitner and Philipp; Skobelzyn in Leningrad; Kikuchi in Tokyo—all within a very few years were operating Wilson cloud chambers with signal success. This was but the beginning of the story, and it is pointless here to continue it. Whoever turns the pages of a physical journal of the past thirty years will see for himself something of what the harvest has been: he will appreciate the abundance of it even more clearly if he turns the pages of "An Atlas of Typical Expansion Chamber Photographs" (1954), with its dedication to "C. T. R. Wilson, whose invention of the cloud chamber made these photographs possible", and its frontispiece reproducing his portrait by James Gunn. There could be no better *Festschrift*.

Wilson gave to physicists in general a sharp tool for research, second to none. By contrast, he gave to very few of them his personal guidance, as director of their individual researches: to Wormell, in the general field of atmospheric electricity (in this notice Wilson's contributions to this field have no more than been hinted); to Powell and Dee and J. G. Wilson in cloud-chamber studies; so the list is almost complete. Too much must not be made of this distinction: whether he be of the select few, or of the many, every physicist who has ever operated a cloud chamber must acknowledge his debt. It cannot be measured in words.

C. T. R. Wilson was born on February 14, 1869. He died on November 15 in the midst of his family. Mrs. Wilson, his son and two daughters survive him.

NORMAN FEATHER

NEWS and VIEWS

British Ceramic Research Association: Dr. A. T. Green, C.B.E.

DR. A. T. GREEN retires from the directorship of the British Ceramic Research Association on December 31, after thirty-eight years service to the ceramic industry, during which time he has established himself as one of the world's leading ceramists. An old boy of Hanley High School, he joined Dr. Mellor at the British Refractories Research Association after the end of the First World War and quickly became associated with work on refractories for the steel and gas industries, and by his incisive attack on the problems he then encountered he laid the foundation for future developments, the benefits of which are still being reflected in the economics of those industries. He was made assistant director in 1931 and succeeded to the directorship on Dr. Mellor's retirement in 1937. After the Second World War, Dr. Green, who had been made O.B.E. for his contribution to the war effort, was charged with the task of uniting the British Refractories Research Association and the British Pottery Research Association to form the British Ceramic Research Association, and he took up the directorship in 1947. The Association's main laboratories in Queens Road, Penkhull, opened in 1951 by the Duke of Edinburgh, were planned under Dr. Green's direction, and the steady output of research papers—more than 400 have been published since 1948—is a tribute to the inspiration with which he has led his team of research workers. Dr. Green has been able to see, before his retirement, the completion of another laboratory block—to be named the Mellor-Green Laboratories—which is to serve the structural clay products branch of the ceramic industry. Dr. Green was made C.B.E. in 1957, and was awarded the degree of D.Sc. *honoris causa* by the University of Leeds in 1949. He is an honorary member of the Institute of Gas Engineers and of the British Ceramic Society; the latter he has served as honorary general secretary for the past twelve years. He was one of the founders of the Institute of Ceramics and was its first president.

Dr. N. F. Astbury

DR. N. F. ASTBURY, who has been deputy director since 1957, will succeed Dr. Green. Dr. Astbury was a scholar of St. John's College, Cambridge, where he obtained first-class honours in both parts of the Natural Sciences Tripos. After leaving Cambridge, Dr. Astbury was for ten years on the staff of the National Physical Laboratory, and at the outbreak of war went to the Anti-Submarine Experimental Establishment. In 1945, he entered industry to become director of research of the firm of Joseph Sankey and Son, Ltd., and then director of research to the Guest Keen Nettlefold Group of Companies, of which Sankeys is a member firm. During this time Dr. Astbury planned the central research laboratory for the group, which has now been established in Wolverhampton for ten years. Since 1949 Dr. Astbury has held two professorships—one, the chair of applied physics in the University of New South Wales and the other in the University of Khartoum, where he was also dean of the Faculty of Science. For a short time before joining the British Ceramic Research Association he was at the Royal Aircraft Establishment, Farnborough. Dr. Astbury was awarded his Sc.D. by the University of Cambridge in 1954. He serves on the Education and Membership Committee of the Institute of Physics and on the Advisory Board of the *Journal of Scientific Instruments* and the *British Journal of Applied Physics*, and he is also a member of the Board of Studies of the National Council for Technological Awards.

Deputy Director of the National Physical Laboratory: Dr. G. Macfarlane

Dr. George Macfarlane, a deputy chief scientific officer at the Royal Radar Establishment, Malvern, has been appointed deputy director of the National Physical Laboratory. He succeeds Dr. Edward Lee, who has occupied the post since March 1958 (see *Nature*, **181**, 600; 1958), and who now becomes director of the Stations and Industry Divisions at the headquarters of the Department of Scientific and Industrial Research.

Dr. Macfarlane, who is forty-three, graduated with first-class honours in electrical engineering at the University of Glasgow in 1937, and went on to do two years postgraduate research at Dresden, where he gained the Dr.Ing. degree in 1939. He joined the Telecommunications Research Establishment (now incorporated in the Royal Radar Establishment) in 1939. Throughout the war years he concentrated on mathematical problems in radar and microwave physics. In 1945, he became head of the Mathematical Group, and a year later took charge of the Theoretical Physics Division. Since 1953 he has been carrying out individual research in the Physics Department of the Establishment.

European Organization for Nuclear Research, Cern

In the fourteenth session of the Cern Council which was held in Geneva on December 2 it was proposed that international co-operation should be developed in the field of high-energy accelerators such as those constructed at the European Centre for Nuclear Research in Geneva. The contributions of the Member States have undergone some modification as a result of the accession of Austria to Cern and of changes in the national incomes.

Country	1960–62 (%)	1957–59 (%)
Austria	1·93	—
Belgium	4·15	4·89
Denmark	1·99	2·23
France	21·22	22·26
German Federal Republic	19·52	18·27
Greece	1·17	1·08
Italy	10·09	10·61
Netherlands	3·85	3·78
Norway	1·61	1·72
Sweden	4·23	4·85
Switzerland	3·29	3·48
United Kingdom	25·00	25·00
Yugoslavia	1·95	1·83
	100	100

The Council approved contributions from the thirteen member States totalling 65,000,000 Swiss francs for 1960. This compares with a budget of 55,000,000 Swiss francs for 1959. Of the 1960 expenditure, 38,500,000 Swiss francs are earmarked for capital expenses, of which 15,000,000 Swiss francs are for new buildings and general site facilities, and 18,000,000 Swiss francs for auxiliary experimental equipment. The remaining 26,500,000 Swiss francs will cover general staff and operating expenditure.

The following appointments were announced: F. de Rose (France) and Prof. W. Heisenberg (Germany) and J. Willems (Belgium) were re-elected respectively president and vice-presidents of the Council. J. H. Bannier (Netherlands) was re-elected chairman of the Finance Committee. Prof. F. Perrin (France) takes the place of Prof. H. Alfvén (Sweden) on the Scientific Policy Committee.

Reactor Project at Halden

The second fuel charge for the Organization for European Economic Co-operation Boiling Heavy Water Reactor Project at Halden, Norway, has been ordered by the Norwegian Institutt for Atomenergi from A.B. Atomenergi of Sweden. The order is worth approximately 500,000 dollars. The Halden reactor, which is one of the two experimental reactors in the current programme of the Organization's European Nuclear Energy Agency, has been in operation at zero power since last June using a fuel charge of natural uranium metal. With this charge, to which it is intended to add a certain number of slightly enriched uranium 'spikes', a maximum power of approximately 5 MW. is expected. The second fuel charge will consist of approximately 1,500 kgm. of 1·5 per cent enriched uranium oxide, made up into 100 7-element assemblies of zircalloy-clad rods. This charge is expected to enable powers up to 20 MW. to be obtained. Delivery of the second charge will be in March 1961, and will include eight extra fuel assemblies, without uranium, for experimental use in the reactor core.

Mental Health Problems in Old Age

A new report issued by the World Health Organization (Technical Report Series, No. 171. Geneva: W.H.O.; London: H.M. Stationery Office. 3*s.* 6*d.*) deals with some of the psychological and social problems posed by the ageing of populations. It is the work of a small expert committee which met in Geneva last year to consider the broad psychiatric implications of present population trends. In its report, the committee stresses particularly the need for integrated mental health services for the aged which would provide guidance as well as medical care. Emphasis is also placed on the need for fundamental research on the psychological assets and disabilities of older people. Although this report deals principally with the strictly psychiatric aspects of old age, it deserves careful study by all concerned with the biology of ageing and the care and management of the elderly.

Friends of the Lake District

The annual report of the Friends of the Lake District reviews the work of the association since it was founded twenty-five years ago. So long ago as 1936 an agreement was reached with the Forestry Commission not to acquire for afforestation any land within the central 300 square miles of the Lake District. In 1937 the first proposals for making the Lake District into a national park were put forward, while, in 1937, the association persuaded the Westmorland and District Electricity Co. to modify its plans about overhead electricity lines and to put them underground. By 1938 membership of the society had reached 2,700. In the same year, a road policy for the Lake District was produced, the society supporting a case against the making-up as motor roads of the remoter mountain passes and urging restraint against the up-grading of Lake District roads generally. At about this time measures were first taken to protect the rarer birds which nest in the high fells. This work has continued over the years with beneficial effects. The society has also taken vigorous action to prevent the silting and discoloration of Ullswater by effluent from the Glenridding lead mine. Other matters in this interesting

report include accounts of the measures taken to mitigate the effects of war training areas, control of war-time factories, and the building of reservoirs by local authorities. The report (Friends of the Lake District. 1934–1959 Retrospect and Annual Report, June 1959. Pp. 30) may be obtained from the Secretary, 14 Princes Street, Ulverston, Lancs.

Freshwater Biological Association

THE now familiar style of the yearly report of the Freshwater Biological Association should be taken as a model of the way in which reports of scientific associations could be presented (Freshwater Biological Association. Twenty-seventh Annual Report for the year ended March 31, 1959. Pp. 81. (Ambleside: Freshwater Biological Association, 1959.) 4*s*.). Although brief, it gives details of the administrative work of the Association during the year, a statement of its finances, a description of the staffing position, as well as a clear account of the scientific work carried out by the staff during the year supported by a list of all papers submitted by them to learned societies and journals. The presentation should be of particular value to lay members of water boards throughout the country. Among interesting scientific work was that by Dr. Talling on the photosynthetic behaviour of plankton algae, considered in relation to the problems of primary production in lakes. Particular attention has been given to the variation of photosynthesis rate with depth, a fundamental relation in calculations of photosynthetic productivity below unit area. In the course of work by Dr. Willoughby on the distribution of the lower saprophytic fungi (Chytridiales and Blastocladiales) of Esthwaite water, a species of *Blastocladiella* has appeared repeatedly in baitings from the marginal zone. The group Blastocladiales is one which has excited a great deal of interest especially in America, but no member of either of the two most important genera, *Allomyces* and *Blastocladiella*, had ever been recorded before in Britain, and no *Blastocladiella* had been reported from Europe. The species found in Esthwaite occurred on all three types of bait used, cellulose, chitin and keratin, and its ability to grow on all three has been verified in cultures grown from a single spore. Unlike *B. emersonii* Cantino, it does not respond to treatment with bicarbonate by producing resting spores. The report is available from the Secretary, The Ferry House, Far Sawrey, Ambleside, Westmorland.

The Institution of Mining Engineers

IT is announced that Mr. R. G. Baker has been elected president for the year 1960–61 in succession to Mr. T. A. Rogers. Ir. A. Hellemans (director, Staatsmijnen in Limburg) and Dr. J. Taylor have been elected honorary members of the Institution. The tenth award of the Douglas Hay Medal, instituted in 1950 in memory of the late Prof. D. Hay, past-president, has been made to Mr. A. E. Crook in recognition of his valuable contributions to engineering practice in mining with special reference to haulage and winding and for his papers on these subjects in the *Transactions*. The Maskell Peace Scholarship of £70 for 1959, the tenth since the Institution accepted the trusteeship of the fund from the Mining Association of Great Britain, has been awarded by the North of England Institute of Mining and Mechanical Engineers to Mr. P. Hutchinson, a student at the Department of Mining Engineering, King's College, University of Durham.

Trevelyan Scholarships

THE first awards of these scholarships were made in 1958 and the second awards have now been made by the Trust. The scheme is sponsored by the following companies: Arthur Guinness Son and Co., Ltd.; Associated Electrical Industries, Ltd.; Courtaulds, Ltd.; English Electric Co., Ltd.; Guest Keen and Nettlefolds, Ltd.; Imperial Tobacco Co., Ltd.; John Summers and Sons, Ltd.; Peninsular and Oriental Steam Navigation Co.; Rolls-Royce, Ltd.; Steel Company of Wales, Ltd.; Stewarts and Lloyds, Ltd.; and the United Steel Companies, Ltd. Sufficient funds have been subscribed to permit the award each year of not less than 34 three-year scholarships of £450 a year over a period of five years. It was arranged that all applications would first be considered at either Oxford or Cambridge, according to the applicants' first choice, the University panels agreeing upon 'short lists' of candidates for final interview by a selection committee. For the second selection 198 applications were accepted, and from these a short list of 58 applicants were interviewed. In 1958, 123 applications were accepted and 54 applicants were interviewed. The selection committee restricted the awards to 23 in number, of which 12 are tenable at Oxford and 11 at Cambridge. In 1958, 19 awards were made: 10 at Oxford and 9 at Cambridge.

Announcements

COMMENCING January 9, a course of twelve lectures and appropriate practical work on micro- and semi-microchemical methods will be held on Saturday mornings at the Norwood Technical College, London, S.E.27. This course has been arranged for teachers, industrial and research chemists, and is designed to survey the principal branches of chemistry in which small-scale methods have been successfully applied. Further information can be obtained from the secretary of the College.

THE Bradford Institute of Technology has announced that it is holding a symposium on "Recent Developments in Textile Chemistry" and also a short course in "New Techniques in High Polymer Chemistry". The symposium is to be held on February 13 in conjunction with the Bradford Chemical Society and the Yorkshire Council for Further Education. The course is to take place during February 26–27. Both are to be held at the Bradford Institute of Technology. Further information can be obtained from the Registrar, Institute of Technology, Bradford 7.

THE Association of Agriculture has announced that a series of eight evening lectures, the third in its kind to be organized in collaboration with the Extra-Mural Department of the University of London, will be held during January 20–March 9, 1960. The series, "The Land of Britain, is it used to the Best Agricultural Advantage?", will take place at the London School of Hygiene and Tropical Medicine, Keppel St., London, W.C.1. Further information can be obtained from the General Secretary, The Association of Agriculture, 53 Victoria St., London, S.W.1.

ERRATUM. The authors of the communication entitled "Mineral Uptake and Retention in Cotton-grass (*Eriophorum vaginatum* L.)" published in *Nature* of August 8, p. 467, state that the ordinate of Fig. 1 was incorrectly printed by them; for "m.eq." read "gm.".

THE BRITISH COTTON INDUSTRY RESEARCH ASSOCIATION

OPEN DAYS AT THE SHIRLEY INSTITUTE

THE fortieth annual general meeting of the British Cotton Industry Research Association was held at the Shirley Institute on October 22, with the chairman of the Association, Mr. N. G. McCulloch, presiding. In his address Mr. McCulloch said that he wished to reiterate a view that he had many times expressed, that there was an urgent need in the Lancashire textile industry for a much more concentrated and sustained study of the subject of organization and management than had so far been attempted. The Shirley Institute was no stranger to this field of work and had made a number of worthy contributions, but the effort needed intensifying and widening. Mr. McCulloch also referred to the working party which had been set up to consider the problems involved in the proposed fusion of the Institute with the British Rayon Research Association, and emphasized the importance of this latest move towards rationalizing co-operative research for the Lancashire textile industry.

Mr. J. Lindley, honorary treasurer, in reviewing the statement of accounts for the year, was particularly concerned to emphasize the need for adequate reserves, since expenditure on research could not be turned either off or on like a tap. He pointed out that the general reserves at present were in fact only sufficient to pay the Association's outgoings for rather less than nine months.

At the end of the meeting, Mr. McCulloch announced his forthcoming resignation and tributes were paid both by Mr. Lindley and from the floor of the meeting to his great contribution as chairman for the past ten years.

Dr. D. W. Hill, director of research, then presented his report on the year's work and stressed the consideration which had been given during the year to the need for a research association to follow up its research work with all possible steps to ensure application in mills and works. This was a particular concern of the Institute during the industry's difficult times, and it was reflected, for example, by an increase of 14 per cent in technical inquiries received by the Institute during the preceding year. There was also a need to keep abreast of industrial advances in foreign countries, to which end four teams had been sent to the Continent to visit Holland, Italy, France and Western Germany and to report their experiences to members.

In selecting a few research and development topics for special comment, Dr. Hill mentioned the continuing work for spinners on control of regularity of their intermediate products, and referred to the success of the cop sorting device for condenser spinners. A new programme of work was now starting on winding and beaming, with particular emphasis on the variations in the density of various wound packages. Results of interest to both spinners and weavers should follow the introduction of a new technique involving the use of a radioactive isotope within the package. The work on sizing continued and attention had been paid to, and a device developed for, reducing the hairiness of yarns, particularly those of synthetic fibres. Work on the dry-feed attachment of the automatic size box had reached the point of initiating mill trials. It was impossible, with the limited resources available, to do simultaneous research on all the widely diverse problems of the industry, and it had been decided to devote attention at intervals to the smaller and more specialized sections. At the moment, for example, a fundamental study of the operation of a terry-towel loom was being started.

On the chemical side, Dr. Hill directed particular attention to past history of work at the Institute on bleaching processes, work which had been recently brought up to date by a thorough examination of the mechanism of sodium chlorite as a bleaching agent, which had also produced very valuable technological results. Also for finishers, Dr. Hill reported that a long-term physical study of the action of the raising machine had resulted in the introduction of a new kind of instrumentation in this process which should be of great technical benefit.

As in previous years, the Institute laboratories were open for inspection for two days, when all members of staff were available for consultation.

Spinners were particularly interested in an automatic nep counter, so far only used for research purposes, in which a photoelectric method is used for detecting neps, followed by an electronic counter. A new version of the Shirley opener was also shown, as was a recent further improvement to the pressure-point system.

In the Weaving Department a large exhibit attracted the attention of members to the widely varied services the Institute could offer them, while another exhibit analysed the major causes of warp breaks during weaving. A stretch regulator, the Shirley loom and the new device for reducing hairiness were also operating. Most interest in this section, however, was raised by the exhibit showing the range of, and methods used in, the investigation being carried out on yarn clearing.

Finishers were given working demonstrations of the flash steamer now commercially available and of the new raising action indicator. A highly important new programme is that on washing efficiency, with the high cost of water and the expense of effluent disposal becoming increasingly serious. Preliminary results already suggest that there will be considerable gains from working in this field.

Fundamental work on the mechanism of the burning of cellulose has started and there were demonstrations both of the production of inflammable tars as intermediate products, and also of the behaviour of existing flame-proofing agents. The use of infra-red absorption analysis for identification of finishes and contaminants was interesting both fundamentally and as an aid to the technologists.

The Institute continues its fundamental investigation of the effect on yarn and fabric properties of blending natural and synthetic fibres, and a general display showed the extent to which it is now possible to forecast many of these properties from a previous knowledge of fibre properties.

Old and new instruments were in evidence both for research and control purposes. As usual, there was a stand showing the instruments being handled

by Shirley Developments, Ltd., which, as a result of rapid growth, now shows in its catalogue 30 instruments and 30 mechanical devices.

New instruments for research purposes included a new photomicrographic apparatus designed and made at the Institute, and also a polarizing lustre meter with which it is hoped to interpret how the eye judges lustre.

It was encouraging that a large proportion of the visitors on the open days came with the definite object in mind of seeing how the Institute's work made an impact on their own mill or works.

AGRICULTURAL INSTITUTE OF THE REPUBLIC OF IRELAND

DIVISION OF PLANT SCIENCES AND CROP HUSBANDRY

THE establishment, under the Agricultural (An Foras Talúntais) Act, 1958, of the Agricultural Institute of the Republic of Ireland, An Foras Talúntais, was announced in *Nature*, July 4, 1959. The Institute now announces major developments in the organization of the Division of Plant Sciences and Crop Husbandry. This will provide a wide coverage in the plant sciences field, catering for research in plant pathology, entomology (including nematology), plant breeding, plant protection, plant quality, weed control, and other general aspects of crop husbandry.

Mr. B. Crombie, at present in charge of the Plant Breeding Station of Cómhlucht Siúicre Éireann, Teo. (Irish Sugar Company), at Thurles, Co. Tipperary, has been appointed chief of this Division. Mr. Crombie, a native of Offaly, gained his B.Agr.Sc. degree with honours at the National University of Ireland in 1934 and was awarded a postgraduate scholarship. In 1937 he gained his M.Sc. degree for a thesis on "The Influence of Various Factors on the Time of Ripening of Wheat Varieties". While working under Prof. M. Caffrey, University College, Dublin, Mr. Crombie was responsible for the breeding of the well-known Glasnevin strains of perennial ryegrass and cocksfoot. He had been responsible for the organization of seed-breeding research and developments with Cómhlucht Siúicre Éireann, Teo., giving to the industry sugar-beet seeds of top quality. Mr. Crombie has published papers dealing with his research work in a number of scientific journals. He comes to his new post with a high reputation not only in plant breeding but also in plant science and crop-husbandry work in general.

By mutual agreement between the Council of An Foras Talúntais and the Industrial Research Committee of the Institute for Industrial Research and Standards, the facilities and equipment of the Cereals Laboratory at the latter Institute have been made available to An Foras Talúntais. This laboratory will devote special attention to the question of baking quality in wheat in relation to soil, manurial, climatic, varietal and cultural conditions, while in addition undertaking research on quality in other cereals. The general supervision of this laboratory for An Foras Talúntais will be in the hands of the director of the Institute for Industrial Research and Standards, Mr. D. T. Flood. Mr. P. A. Spillane has been appointed a senior research officer on the staff of An Foras Talúntais and will be head of the laboratory. Other appointments to this Unit are those of Miss H. McGovern as research officer and Misses K. McGrath and E. Cooper as technicians. Mr. Spillane graduated from University College, Dublin, with honours in physics and chemistry in 1936. He has a wide experience of research and general operational work in the flour-milling industry and is the author of a number of scientific papers dealing with this work.

It has been agreed in principle between the Board of Cómhlucht Siúicre Éireann, Teo., and the Council of An Foras Talúntais that the sugar-beet breeding research facilities at Thurles and the Nematology Laboratory at Carlow of the Irish Sugar Company will be integrated with An Foras Talúntais. These centres have already been placed under the scientific direction of An Foras Talúntais pending the conclusion of arrangements by the staffs of the organizations concerned. This arrangement places at the disposal of the Agricultural Institute the scientific staff and extensive offices, laboratory and greenhouse facilities and equipment which have been developed at these centres over a number of years. While the sugar-beet seed research programme will be continued and expanded at Thurles by An Foras Talúntais on behalf of Cómhlucht Siúicre Éireann, Teo., this centre will also act as headquarters of the Division, with the resulting development of research services in general plant science work apart from those needed for sugar-beet research.

The nematology laboratory at Carlow which provides well-developed facilities for this sphere of research will also be used in the general research programme of the Institute. Dr. J. J. Duggan has been appointed senior research officer in charge of the Nematology Unit of the Institute now located at University College, Dublin. Dr. Duggan is well known for his research work in nematology on which he has published a number of scientific papers, and the Unit at Carlow is now under his scientific direction.

A Plant Pathology Unit has been established. Dr. J. B. Loughnane, Department of Plant Pathology, University College, Dublin, is acting as adviser for this aspect of the activities of the Institute. Mr. P. Cunningham and Mr. C. Comerford are now working under Dr. Loughnane in the Plant Pathology Unit, while Mr. T. E. Mulligan, previously on the staff of the Rothamsted Experimental Station, joined the staff of the Unit on October 1. Associated Seed Growers, Ltd., have generously offered a grant for the investigation of the diseases of a number of crops such as kale and mangels. This offer has been accepted by the Council of the Institute and investigations are already under way.

Arrangements are also being completed for placing an Applied Plant Genetics Research Unit at the Kells Ingram Farm of the University of Dublin, working under the general direction of Mr. G. W. P. Dawson, head of the new Department of Genetics, Trinity College, Dublin. This Unit will deal with some of the more practical aspects of plant genetics. Mr. V. Connolly has been appointed research officer in this Division. Arrangements are proceeding with the organization of the other departments of the Division, and it is hoped to have a full research programme under way during the coming year.

ELECTROPHORESIS OF HUMAN GASTRIC JUICE

By Dr. RAGNHILD GULLBERG and Dr. BÖRJE OLHAGEN
Konung Gustaf V:s Forskningsinstitut, Stockholm 60

ANALYSIS of the macromolecular components of gastric juice has hitherto given varying results not readily comparable[1-5]. This is in all likelihood due to defective control of autodigestion. The proteolytic effect of the pepsin is so rapid at the low hydrogen ion concentration generally prevailing in normal gastric juice that it is not sufficient to neutralize the juice immediately after aspiration. For that reason, we attempted to neutralize the gastric juice already in the stomach by introducing sodium phosphate buffer.

The gastric juice was collected as a fasting secretion or after insulin stimulation. A suction device prevented the admixture of saliva. Samples contaminated with blood or bile were discarded. The juice collected was cooled immediately on ice and treated continuously in the cold. Visible mucus was eliminated by centrifugation or filtration through glass wool. The juice was concentrated by ultrafiltration through collodion membrane. Paper electrophoresis was performed according to Glass *et al.*[1] in borate buffer at a *p*H of 9·0.

Gastric juice which had been neutralized in the stomach with sodium phosphate buffer of varying *p*H and ionic strength 0·2 was compared with further samples from the same individual, one neutralized immediately after aspiration and another not neutralized. In addition, juice that had been neutralized in the stomach was compared with portions of the same sample which after neutralization had been acidified with 0·1 *N* hydrochloric acid to varying *p*H levels at different temperatures and for varying periods and then again neutralized.

Three main components were found to stain with amido black in 20 human gastric juice samples neutralized in the stomach (Fig. 1). The most rapidly

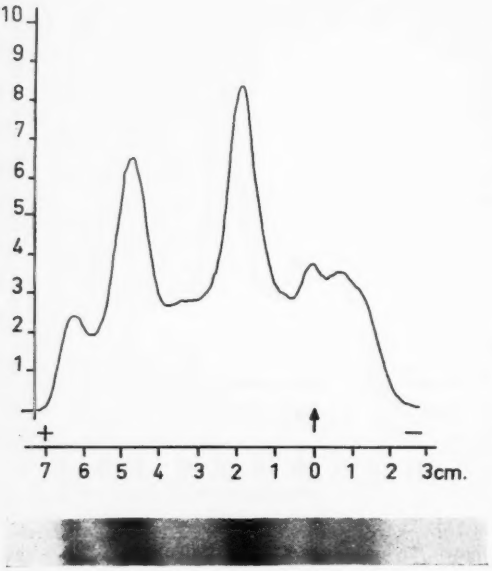

Fig. 1. Paper electropherogram of normal gastric juice neutralized in the stomach with sodium phosphate buffer to *p*H 7·2. Amido black stain

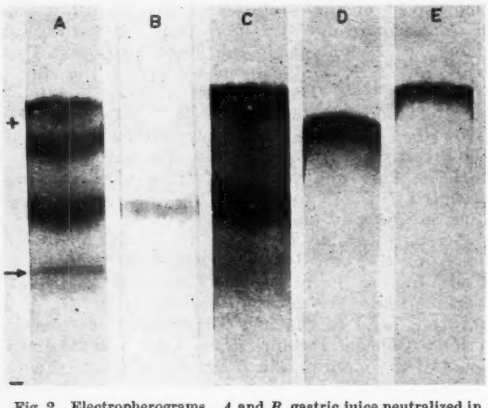

Fig. 2. Electropherograms. *A* and *B*, gastric juice neutralized in the stomach, stained with amido black and periodic acid–Schiff respectively; *C*, same juice aspirated after neutralization in the stomach and acidified with 0·1 *N* hydrochloric acid for 5 min. at 24° C. (*p*H 1–2); *D*, human serum albumin; *E*, crystalline pepsin

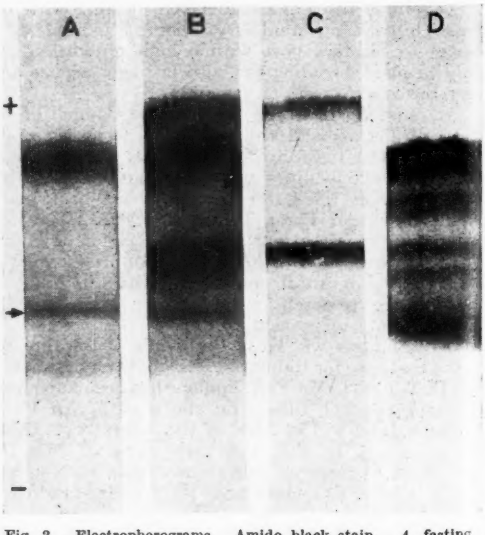

Fig. 3. Electropherograms. Amido black stain. *A*, fasting secretion, *p*H 6. The pepsin band is missing; *B*, gastric juice from the same individual after insulin stimulation, neutralized in the stomach to *p*H 7·2; *C*, juice from the same individual, not neutralized, *p*H 1–2—the albumin is missing; *D*, serum pattern, borate buffer *p*H 9·0

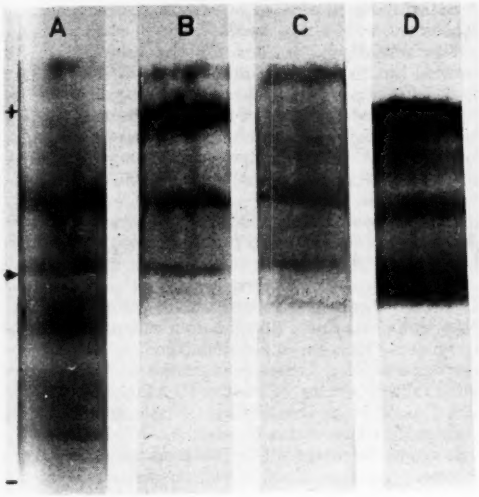

Fig. 4. Electropherograms. Amido black stain. *A*, fasting secretion, not neutralized, *p*H 2–3; *B*, gastric juice from same individual, neutralized in the stomach to *p*H 7·2; *C*, same juice aspirated after neutralization in the stomach and acidified with 0·1 *N* hydrochloric acid for 2 min. at 24° C. (*p*H 1–2)—disappearance of the albumin band; *D*, serum pattern, borate buffer *p*H 9·0

anodically migrating component has the same mobility as crystalline pepsin (Fig. 2*E*) and has earlier been shown to be a carrier of peptic activity[1]. It is not present in gastric juice from achylia patients (Fig. 5*A*) or in non-acid fasting secretion (Fig. 3*A*); it is occasionally absent in juice which has been neutralized in the stomach to *p*H 8·0, and is in any event less readily stained than when the juice is neutralized to a *p*H of 7·2.

The mobility of the second component corresponds to that of pure serum albumin (Fig. 2*D*). This fraction is invariably present also in gastric juice from achylia patients (Fig. 5*A*), while it is absent or found in only small amounts in acid juice not neutralized in the stomach (Figs. 3*C*, 4*A*) and in juice neutralized in the stomach and afterwards acidified (Figs. 2*C*, 4*C*). It persists after acidification of achylia juice to *p*H 1–2. Gel diffusion analysis according to Ouchterlony[6] exhibited a heavy immunoprecipitate against anti-albumin serum. It is of interest that even normal human gastric juice contains a protein component which seems to be identical with serum albumin, especially in view of recent studies which have shown a leakage of large amounts of albumin into the stomach in so-called idiopathic hypoproteinæmia[2,7].

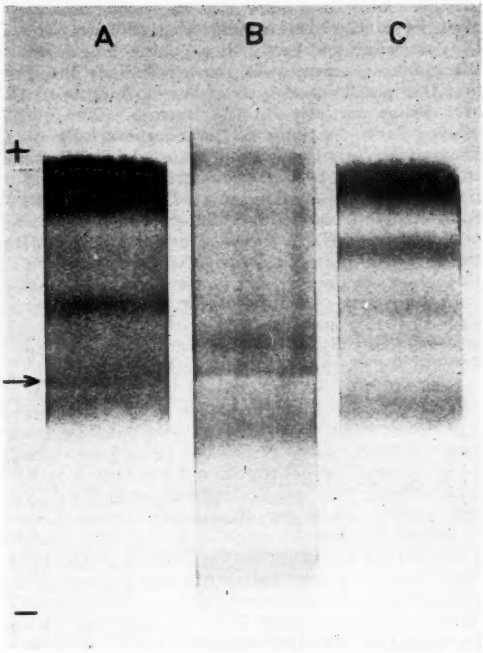

Fig. 5. *A*, Achylia gastric juice; *B*, same juice incubated at 37° C. with crystalline pepsin at *p*H 1–2 for about 5 min.; *C*, serum pattern, borate buffer *p*H 9·0

The third component shows a high carbohydrate content with periodic acid–Schiff staining and may, accordingly, represent a mucoprotein complex (Fig. 2*B*).

Acid gastric juice not neutralized in the stomach contains from 2 (Fig. 3*C*) to 8 components, some of which are strongly basic and appear on the cathodic side of the paper strip (Fig. 4*A*). A similar electrophoretic pattern is found in achylia gastric juice incubated with pepsin at a low *p*H (Fig. 5*B*). Our investigation would indicate that the majority of the many fractions usually found in acid gastric juice, not neutralized in the stomach before aspiration, are breakdown products.

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ECOLOGY OF ACRANIATE LARVÆ

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LITTLE is known of the habits of acraniate larvæ; the evidence available is conflicting. Willey[1] stated that the larvæ of *Branchiostoma lanceolatum* (Pallas) were entirely planktonic until metamorphosis; van Wijhe[2] suggested that they lived chiefly upon the sea bottom; Webb[3] found that the larvæ of *B. nigeriense* Webb lived part of their life upon the sea bottom, and part in the upper water. It is, therefore, not certain whether the typical acraniate larva spends its life on or near the sea bottom, in the surface layers, or a part in either situation. More-

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over, it seems that there may be two different types of larva in the group, each with its own ecology: the 'amphioxides' larva, living for a long period in the plankton and growing to a relatively large size, and the 'amphioxus' type of larva which is smaller, and living partially on the bottom. The 'amphioxides' larva appears to be characteristic of the Epigonichthyidae, the 'amphioxus', of the Branchiostomidae[4]. The present account records observations upon four species of acraniate larvæ found in the Indo-Malayan plankton, which give some indication of the habits of both types of larva, and which show that the 'amphioxides' larva is also found in the Branchiostomidae. These larvæ were taken during a plankton survey made by the Singapore Regional Fisheries Research Station; they occurred at 3 stations only (namely, Permanent Station No. 5; Station *C*3/17, and Station *C*6/18).

Station No. 5. (Singapore Strait: 1° 14′ N., 103° 55′ E.) At this station diurnal vertical migration was investigated by taking 16 horizontal samples at four depths (surface, 27, 55 and 82 m.) throughout a year. At the last depth, the net was only 6 in. above the bottom. The samples were taken in full daylight, just before sunset, just after sunset and in darkness.

Acraniate larvæ were found regularly in these samples from the latter half of October up to January. They were at various stages in development; some individuals were metamorphosing. These larvæ had 10–21 gill slits and were 3·6–5·2 mm. long. A single juvenile 17·5 mm. long was also found. All belonged to *Branchiostoma belcheri* Gray. Webb[5] has recorded the adult from this area. Analysis of the samples showed that, while numbers of the larvæ were small, their behaviour was consistent. Fig. 1 illustrates the way in which the larvæ stay on, near, or possibly within the sea-bed during the day, and move upward at dusk, showing the largest concentration at the surface for a short period after sunset; afterwards they disperse. As a rule, the greatest numbers of larvæ were taken at night. Bedford[6] found larvæ in Singapore Strait at night only.

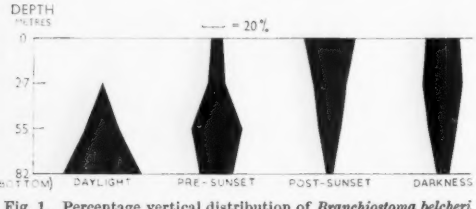

Fig. 1. Percentage vertical distribution of *Branchiostoma belcheri* larvæ

Webb[3] recorded vertical movements of *B. nigeriense* larvæ, and demonstrated experimentally that they reacted to temperature and salinity variations, these responses determining their position in the water layers. At Station No. 5, temperature and salinity variations with depth were negligible, and the larvæ were not being stimulated to move from unfavourable to favourable surroundings as Webb found with *B. nigeriense*. The possibility of passive swirling by up-currents can be discounted. The conclusion is that *B. belcheri* larvæ migrate actively upward through the water, reaching the surface as darkness falls. Twilight at Singapore is short, the two series of samples taken just before and just after sunset being only about 75 min. apart. From this, and the distances involved, it is estimated that the larvæ are capable of moving through a vertical distance of 35 m. in approximately 90 min., equivalent to 6·5 mm./sec.

The actuating stimulus for the migration is not known, but in view of Chin's[7] observations on larvæ of *B. belcheri*, it would appear to be light intensity.

The possibility that the migration is one where the larvæ move into areas richer in food is not suggested by examination of the gut contents. Of the guts examined, 5 (8 per cent) had no discernible food present; the remainder contained food particles in a mucous rope. The following diatom genera were identified: *Coscinodiscus; Pleurosigma; Nitzschia; Melosira; Achnanthus; Navicula; Rhizosolenia* and (?) *Cocconeis*. There were also silico-flagellate skeletons, what are probably small echinoderm spine fragments, and finely divided debris, both organic and inorganic. Most of the diatom genera are normally bottom dwelling; these formed the bulk of the food. No doubt frustules of the two genera *Coscinodiscus* and *Rhizosolenia* would form a natural part of the bottom detritus at this depth.

It seems then that the larvæ feed only when on the bottom; such an intermittent pattern of feeding has been inferred for larvæ of *B. lanceolatum*[8], the cycle taking at least 9 hr. to complete.

A study of the water movements of the area has shown that the diurnal migration would have the effect of distributing the species; it was Goldschmidt[9] who first suggested that each species of acraniate might possess the two types of larva known in the group, and that one would be distributory and pelagic (the 'amphioxides' larva), the other bottom-living; however, the larva of *B. belcheri* is not of the 'amphioxides' type.

Another result of the vertical movement would be the avoidance of nocturnal bottom-feeding predators, for example, penaeid prawns. Mr. D. N. F. Hall (personal communication) informs us that these animals find their food by 'grubbing around' on the bottom, and, although somewhat selective in their feeding habits, many are omnivorous and quite capable of including the larvæ in their diet. These prawns are sufficiently numerous to comb a large area of the sea-bed at night. No doubt other predators behave similarly.

There are other observations that acraniate larvæ of different species rise to the surface at night. Gibson[10] noted that more than three-quarters of his material had been taken at the surface by night. Recent catches of *B. lanceolatum* larvæ in the North Sea have been made at night and just after sunrise at the surface (Cattley, J. G., personal communication, 1957, and Williamson, M. H., personal communication, 1959). It seems, therefore, that the diurnal vertical migrations of *B. belcheri* larva at Station No. 5 are possibly characteristic of acraniate larvæ in general, the behaviour patterns described by Willey and by Webb for *B. lanceolatum* and *B. nigeriense* being perhaps referable to the special environments of each of the populations studied.

*Station C*3/17 (Malacca Strait: 7° 12′ N., 97° 12′ E.). A single sample was collected at this station between 2100 and 2115 hr. (sunset about 1845 hr.), at a depth of 1·5 m. Total depth was 1,005 m. (550 fathoms), the station being about 50 miles outside the 100-fathom line. Larvæ of two species were found; the majority belonged to *Branchiostoma malayana* Webb, but there were also a few of an 'amphioxides' type. The guts of the *B. malayana* larvæ were generally almost empty, but some larvæ had caught relatively large frustules of *Rhizosolenia* sp., which

lay along the peripharyngeal bands and at the top of the branchial chamber. More common were chain diatoms (probably *Bellerochea* sp.), which were sometimes longer than half the entire branchial chamber. One gut contained part of a single small crustacean limb appendage and a doubtful small crustacean antenna; these were not lying along the top of the branchial chamber. Both forms of diatoms are normally pelagic, suggesting that these larvæ had been feeding in mid-water, and in any event, the depth of water over which the larvæ were caught does not suggest that they can have been feeding near the bottom. From the position of the station, the depth of water and the gut contents of the larvæ, it appears that the 'amphioxus' type of larva of *B. malayana* is truly pelagic, and that it does not spend the daylight hours upon the bottom as does the larva of *B. belcheri*. No metamorphosing individuals were found; the larvæ had 12–18 gill slits. Only two adults of this species are known, both collected by one of us from the inter-tidal zone of south-east Singapore[5].

The amphioxides larvæ collected at this station belong to the Branchiostomidae, forming the second record of this type of larva from the family (Fuller[11]). They are of a different species from that discussed by Fuller, and will be described in a subsequent publication.

Station C6/18 (800 miles E.N.E. Singapore: 6° 18′ 25″ N., 115° 36′ 50″ E.). Only 3 acraniates were collected from this station, one a post-metamorphic *B. belcheri*, the others amphioxides larvæ, probably belonging to *Amphioxides pelagicus* (Gunther), an entirely pelagic form and almost certainly the larva of *Asymmetron lucayanum* Andrews.

Goldschmidt[12] stated that doubt remained because the form of the tail fin in his specimens of *A. pelagicus* differed from that of the *Asymmetron* adults; it is interesting that the present larvæ show a tail fin more similar to the adult than did Goldschmidt's specimens.

Station *C6/18* was situated near a steeply shelving bottom in a depth of 64 m., so that it is not surprising that both pelagic and diurnally bottom-dwelling larvæ were found together.

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ENZYMIC ACTIVITIES OF HUMAN SKELETAL MUSCLE MITOCHONDRIA: A TOOL IN CLINICAL METABOLIC RESEARCH

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MITOCHONDRIA are the well-established site of cellular respiration and of those enzymic processes by which the energy liberated in terminal electron transport is conserved in the form of high-energy phosphate, notably adenosine triphosphate. In intact mitochondria, respiration and the accompanying phosphorylation are coupled together in an obligatory manner, and this coupling is generally considered to constitute the level at which the metabolic rate of the animal organism as a whole is regulated. In spite of the vast amount of experimental work with isolated mitochondria which underlies these concepts, relatively few studies are so far available on mitochondria from skeletal muscle, the largest tissue of the vertebrate body, and none, to our knowledge, on skeletal muscle mitochondria from human beings. Considering the relative simplicity with which specimens of human skeletal muscle can be obtained by biopsy, such studies were felt to be of some interest, with potentialities of providing information of both biochemical and clinical significance.

In the present work, some enzymic properties of normal human skeletal muscle mitochondria were investigated and compared with those of mitochondria from a choice of patients with elevated basal metabolic rates. The following well-known characteristic properties of intact vertebrate mitochondria were considered: their capacity to catalyse the aerobic oxidation of various Krebs cycle metabolites and glutamate[1]; to carry out oxidative phosphorylation[1]; to adjust their respiratory rate to the availability of a terminal phosphate acceptor[2], that is, to display so-called respiratory control; and to exhibit adenosine triphosphatase activity induced by 2,4-dinitrophenol[3].

For the present study, three cases of hypermetabolism were selected: (1) a case of classical thyrotoxicosis of moderate degree (basal metabolic rate + 50 per cent); (2) a patient with so-called thyrotoxicosis factitia, that is, a subject deliberately consuming large doses of thyroid hormone (basal metabolic rate + 50–80 per cent); and (3) a patient with an extremely high basal metabolic rate (basal metabolic rate + 150– + 170 per cent) of unknown origin, with no demonstrable thyroid disorder; a detailed description of this unique case will be published elsewhere.

Specimens of skeletal muscle from subjects with no evidence of metabolic disorder ('normal' subjects) were obtained in connexion with surgical interventions, and from the patients with hypermetabolism by biopsy. In case 3, two biopsies were made, with an interval of sixteen days. The form of anæsthesia, general or local, was shown to be of no significance for the mitochondrial properties studied here.

The mitochondria were isolated according to the well-documented procedure of Chappell and Perry[4], using a homogenizing medium containing 0·1 *M* potassium chloride, 0·05 *M* *tris*-(hydroxymethyl)-

Table 1. Respiration, Phosphorylation and Adenosine Triphosphatase Activity of Skeletal Muscle Mitochondria from Normal and Hypermetabolic Subjects

Case No.	Muscle	Respiration (μatoms oxygen/20 min./gm. muscle) Without hexokinase-glucose	With hexokinase-glucose	Stimulation by hexokinase-glucose (times)	Phosphorylation (μmol. phosphate per μatoms oxygen)	Adenosine triphosphatase activity (μmol. phosphate/20 min./gm. muscle) Without 2,4-dinitrophenol	With 2,4-dinitrophenol	Stimulation by 2,4-dinitrophenol (times)
	Normal subjects							
1	Sartorius	2·1	8·8	4·2	2·9	2·1	12·0	5·7
2	,,	2·4	9·9	4·1	2·7			
3	Pect. major	0·3	4·9	16·3	2·4	4·6	16·8	3·7
4	,,	0·3	3·6	12·0	3·0	4·4	18·0	4·1
5	,,	2·0	9·1	4·6	2·6			
6	Ext. obl. abd.	0·7	6·2	9·0	2·9	4·0	10·9	2·7
7	,,	1·6	6·6	4·1	2·6			
8	,,	1·0	4·1	4·1				
	Hypermetabolic subjects							
1	Sartorius	2·8	6·8	2·4	2·9	4·0	13·5	3·4
2	,,	2·6	3·7	1·4	1·9	6·6	9·0	1·4
3*a*	,,	7·3	8·9	1·2	2·6	9·7	11·2	1·1
3*b*	,,	3·1	4·6	1·5	1·7	11·2	17·7	1·6

For experimental conditions and description of hypermetabolic cases, see text. *a* and *b* refer to two different biopsies made on the same case.

aminomethane buffer, *p*H 7·4, 0·001 *M* sodium adenosine triphosphate, 0·005 *M* magnesium sulphate and 0·001 *M* ethylenediaminetetraacetate ('*tris*-potassium chloride medium'). The conventional sucrose-procedure proved to be unsuitable because of gel-formation during homogenization. The excised muscle-specimens were quickly immersed in ice-chilled *tris*-potassium chloride medium, cut into small pieces and homogenized in 3–4 volumes of *tris*-potassium chloride medium by means of an all-glass Potter–Elvehjem homogenizer. All operations were performed at 0–2° C. The homogenate was diluted with *tris*-potassium chloride medium to contain 1 gm. muscle per 10 ml., and centrifuged in a Model *L* Spinco ultracentrifuge, rotor 30, with 2,500 r.p.m. for 5 min. to remove nuclei and myofibrils. The supernatant was decanted and recentrifuged in the same rotor with 10,000 r.p.m. for 10 min. The resulting sediment, representing the mitochondrial fraction, was resuspended in *tris*-potassium chloride medium and recentrifuged as above. The washing medium was discarded, and the surface of the tightly packed pellet was rinsed several times with 0·25 *M* sucrose in order to remove the last drops of *tris*-potassium chloride medium. The pellet was finally suspended in 0·25 *M* sucrose to contain mitochondria from 1 gram muscle per ml. of suspension.

Respiration was measured by the conventional Warburg method, with air as the gas-phase, and with 0·2 ml. 2 *M* potassium hydroxide in the centre wells. Each manometer-vessel contained mitochondria from 1 gram muscle, 10 μmol. substrate (potassium salt), 50 μmol. potassium phosphate (*p*H 7·5), 3 μmol. disodium adenosine triphosphate, 8 μmol. magnesium chloride, 250 μmol. sucrose, and where indicated, 50 μmol. glucose and an excess of yeast hexokinase, in a final volume of 2 ml. The temperature was 30° C. and the vessels were thermo-equilibrated for 5 min. Phosphate uptake was estimated by the isotope distribution method described by Lindberg and Ernster[5]. The phosphate/oxygen ratio was calculated by dividing the phosphate uptake in μmol. during 25 min. by the 20-min. oxygen-consumption value in microatoms, corrected by extrapolation for the 5-min. period of thermo-equilibration. The test-system for adenosine triphosphatase contained mitochondria from 200 mgm. muscle, 10 μmol. disodium adenosine triphosphate, 10 μmol. *tris*-(hydroxymethyl) aminomethane buffer (*p*H 7·5), 90 μmol. potassium chloride, and 250 μmol. sucrose, in a final volume of 2 ml. 2,4-Dinitrophenol was present, when indicated, in a final concentration of 0·1 m*M*. The samples were incubated in open tubes with gentle shaking at 30° C. for 20 min. After fixation with perchloric acid, inorganic phosphate was estimated by the modified Martin and Doty method[5].

Fig. 1 compares the respiration obtained with various substrates in skeletal muscle mitochondria from a normal and a hypermetabolic (case 3, first biopsy) subject. Both preparations catalysed the oxidation of various Krebs cycle metabolites and of glutamate according to similar patterns. Although the absolute rates of respiration varied to some extent from one preparation to another (Table 1), the relative rates obtained with the various substrates were fairly consistent and independent of the species of skeletal muscle used; moreover, a similar picture was also found with skeletal muscle mitochondria from rat. Pyruvate (in the presence of malate) and α-keto-glutarate were oxidized at relatively high rates, whereas other Krebs cycle metabolites, including pyruvate or malate alone, were oxidized only slowly; the rate of oxidation of glutamate varied between high and medium-high from one preparation to another. Of some interest may be the relatively slow rate at which succinate was oxidized. This finding was in sharp contrast to the case found with mitochondria from other vertebrate tissues, including

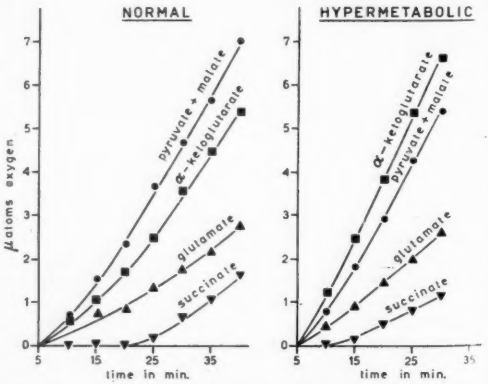

Fig. 1. Respiration of skeletal muscle mitochondria in the presence of various substrates. Muscle specimens (in reference to Table 1): normal subject, case 3; hypermetabolic subject, case 3*a*. For experimental conditions, see text. Hexokinase and glucose were added to all samples

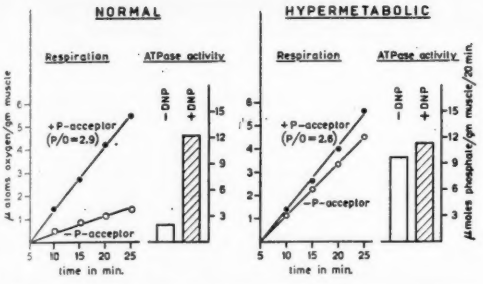

Fig. 2. Respiratory control, phosphorylative efficiency and adenosine triphosphatase activity of preparations of human skeletal muscle mitochondria. Muscle specimens (in reference to Table 1): normal subject, case 1; hypermetabolic subject, case 3*b*. For experimental conditions, see text. Substrate: pyruvate + malate. Hexokinase and glucose were added to samples marked '+ P-acceptor'. DNP = 2,4-dinitrophenol

heart muscle sarcosomes. Glycerol-1-phosphate, which is oxidized at an extremely high rate in insect flight muscle mitochondria[6], was oxidized only very slowly, if at all, in the present preparations. In general, the data presently available give the impression that, in the case of vertebrate skeletal muscle mitochondria, the two α-keto acids are the only Krebs cycle metabolites with a free access to the mitochondrial enzymic sites. In none of these respects were there any differences between the mitochondria from the normal and hypermetabolic groups. Furthermore, addition of diphosphopyridine nucleotide resulted in no increase in the respiration (with pyruvate + malate as substrate), indicating that the mitochondria from both the normal and hypermetabolic subjects had an adequate complement of bound diphosphopyridine nucleotide.

The capacity to respiratory control was tested according to Lardy and Wellman[2], by measuring the increase in respiratory rate occurring upon the addition of an excess of yeast hexokinase and glucose in the presence of a catalytic amount of adenosine triphosphate. Glucose serves in this system as a terminal phosphate acceptor, removing, by way of the hexokinase-reaction, the adenosine triphosphate formed during respiration, and thus continuously regenerating adenosine diphosphate. In carefully prepared mitochondria from most vertebrate tissues, respiration is tightly coupled to phosphorylation, and therefore, a continuous supply of adenosine diphosphate is necessary to maintain a maximum level of respiration. As seen in Fig. 2, the skeletal muscle mitochondria from a normal and a hypermetabolic (case 3, second biopsy) subject revealed a marked difference in this respect. In the normal case, the respiration obtained in the absence of a terminal phosphate acceptor was relatively low and was increased to a considerable extent—by more than 300 per cent—by the addition of hexokinase and glucose. The hypermetabolic mitochondria, on the other hand, exhibited a nearly maximal rate of respiration even in the absence of a terminal phosphate acceptor, and hexokinase and glucose stimulated it only by about 20 per cent. By sharp contrast, both mitochondrial preparations revealed normal phosphorylative efficiencies, the phosphate/oxygen ratios in the presence of hexokinase and glucose being 2·9 in the normal and 2·6 in the hypermetabolic case, as compared with the theoretical maximum of 3. This, it appears that while both preparations were capable of coupling respiration to phosphorylation to a normal extent, this coupling was markedly looser in the hypermetabolic than in the normal mitochondria.

Another means of demonstrating this difference between the two preparations with respect to the tightness of the coupling of respiration to phosphorylation has been to investigate their adenosine triphosphatase activities in the absence and presence of 2,4-dinitrophenol. It is a well-established fact that carefully prepared mitochondria exhibit a very low adenosine triphosphatase activity and that this activity can be greatly stimulated by a concentration of dinitrophenol that uncouples phosphorylation from respiration[3]. As shown in Fig. 2, the mitochondria from the normal-metabolic subject followed this pattern in exhibiting a relatively low endogenous adenosine triphosphatase activity, which could be stimulated 5·7-fold by 0·1 m*M* dinitrophenol; this value is in good agreement with that reported by Chappell and Perry[4] for pigeon breast muscle mitochondria. The mitochondria from the hypermetabolic subject, on the other hand, exhibited a relatively high endogenous adenosine triphosphatase activity, and the stimulatory effect of dinitrophenol was only 1·1-fold. It is noteworthy that the endogenous adenosine triphosphatase of the hypermetabolic subject's mitochondria did not require magnesium ions as an activator, in contrast to the adenosine triphosphatase that can be elicited in isolated normal mitochondria by exposure to conditions which cause structural alteration, for example, ageing[3]. That the activity of adenosine triphosphatase was truly endogenous and not due to contaminating myosin could be ascertained by showing that it was greatly inhibited, to the extent of 85 per cent, by 3·5 m*M* atebrin. As recently shown by Löw[7], this compound, in the concentration mentioned, almost completely inhibits the endogenous mitochondrial adenosine triphosphatase but leaves myosin-adenosine triphosphatase unaffected.

In Table 1, cumulative data are shown regarding respiratory rate, phosphate/oxygen ratio, respiratory control, and dinitrophenol-stimulability of adenosine triphosphatase, from a number of normal cases and the three hypermetabolic cases previously described. Pyruvate + malate was used as substrate throughout these experiments. The two groups differed consistently with respect to the extents of respiratory control, the stimulation of respiration by hexokinase and glucose varying between 4·1- and 16·3-fold in the normal, and between 1·2- and 2·4-fold in the hypermetabolic group. Significant, though less marked, was the difference in the dinitrophenol-stimulation of the adenosine triphosphatase, which was 2·7- to 5·7-fold in the normal group and 1·1- to 3·4-fold in the hypermetabolic group. The phosphate/oxygen ratios were, as an average, somewhat lower in the hypermetabolic than in the normal cases, but, as cases 1 and 3*a* show, virtually normal phosphate/oxygen ratios could definitely be obtained in mitochondria with a markedly low respiratory control. Finally, the respiratory rates per gram muscle varied somewhat from one experiment to another, and so did also the maximum (dinitrophenol-stimulated) rates of the adenosine triphosphatase reaction. The reasons for these variations, which were equally present in the normal and hypermetabolic cases, are being investigated.

The concept that the mitochondrial respiratory control may constitute the biochemical basis of thyroid hormone action was first formulated by

Lardy[8]. This effect has often been interpreted as a secondary consequence of a lowered phosphorylative efficiency[9], a deficient complement of bound mitochondrial co-factors, notably diphosphopyridine nucleotide[10], or a swollen mitochondrial structure[11]. Yet, Lipmann *et al.*[12] were able to demonstrate a normal phosphorylation and no deficiency of diphosphopyridine nucleotide when liver mitochondria from thyrotoxic animals were incubated under appropriate conditions.

The present results support the concept that a deficient control of respiration by available phosphate acceptors, paralleled by an increased endogenous adenosine triphosphatase activity, constitutes the primary manifestation of the hypermetabolic state at the mitochondrial level. The data seem clearly to eliminate the possibility of these changes being secondary to other alterations of the mitochondrial enzyme organization of the types mentioned above, at least as far as a lowered phosphorylative efficiency and a deficient complement of bound co-factors, such as diphosphopyridine nucleotide and magnesium, are concerned. Furthermore, according to preliminary data, these mitochondria reveal no increased tendency to swelling; the fine structure of the mitochondria *in situ* is being investigated by electron microscopy. Finally, the results also seem to indicate that this alteration of the mitochondrial function may be present regardless the etiology of the hypermetabolism, and that, as case 3 indicates, a thyroid disorder is no prerequisite of the mitochondrial dysfunction described in the present work.

Recently, Remmert and Lehninger[13] reported the isolation from mechanically disrupted mitochondria of a protein factor which, when added to phosphorylating mitochondrial fragments, abolished the respiratory control without appreciably affecting the phosphate/oxygen ratio. The release of this factor from the mitochondria may, according to these authors, be subject to physiological or endocrine control, determining the state of respiratory control in the cell. The possible involvement of Remmert and Lehninger's factor in the present connexions is being investigated.

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SEROLOGICAL DISTINCTION OF ADULT AND FŒTAL HUMAN HÆMOGLOBIN

By G. R. E. NAYLOR and Mrs. M. E. ADAIR

Department of Pathology and Physiological Laboratory, Cambridge

THE interpretation of serological studies of hæmoglobin is difficult because a preparation of hæmoglobin may contain two or more distinct hæmoglobins as well as other soluble antigenic constituents of the red cell. Markowitz, Cartwright and Wintrobe[1] have investigated quantitatively antisera prepared against erythrocuprein. Among the anti-hæmoglobin sera used in the present work several contained anti-carbonic anhydrase. Boivin and Hartmann[2] found several antigens in the hæmoglobin solutions used in their serological studies; they obtained evidence that two of the antigens in normal adult red cells were hæmoglobins. Ruggieri and Marchi[3] have also presented serological evidence of two hæmoglobins in preparations from adult red cells and considered one of them to be fœtal hæmoglobin.

In the present work, serological differences between hæmoglobin from adult human blood and from umbilical-cord blood have been demonstrated by Oakley and Fulthorpe's agar diffusion technique[4]. Antisera were obtained from rabbits which received intravenous injections of adult human hæmoglobin without adjuvant. The antiserum used for the experiments shown in Figs. 1 and 2 and Table 1 gave a red precipitate containing 5·08 mgm. protein which included 0·47 mgm. hæmoglobin when 1 ml. antiserum was mixed with homologous antigen in slight antiserum excess of the optimal proportions[5].

In agar diffusion experiments, antiserum was mixed with an equal volume of 2 per cent agar at 50° C.; the mixture was put in a tube of 6·5 mm. calibre to make a column 18 mm. long and allowed to solidify. 1 per cent agar was added to make a column 17 mm. long and, after this had solidified, 0·3 ml. of 0·16 per cent hæmoglobin was added. Agar and antigens

Table 1

Hæmoglobin antigen number	Distance in mm. between lower boundaries of two precipitation bands in agar after 11 days	Optimal proportions titrations	
		Optimal ratio with 1 per cent antigen	Flocculation time of optimal mixture with antiserum diluted 1 in 25
Adult 1	—	1 : 15	4 min. 45 sec.
Cord blood 2	3·7	1 : 22	17 min. 30 sec.
Cord blood 3	2·9	1 : 23	13 min. 30 sec.
Cord blood 4	2·0	1 : 21	12 min. 30 sec.
Cord blood 5	1·2	1 : 19	8 min. 15 sec.

were dissolved in 0·85 per cent sodium chloride; 1 in 10,000 merthiolate was present in each reagent and the *p*H of the antigens and of the 1 per cent agar was adjusted to about 7·8 by addition of sodium bicarbonate.

Fig. 1 shows the reaction observed after eleven days at room temperature. In each tube antigen and antibody have diffused into the middle layer, which consists of agar only, and a band (really a disk) or bands of precipitate have formed in this layer. Antigen 1, which was hæmoglobin prepared from adult human blood, showed one band of red precipitate. Antigens 2, 3, 4 and 5 were hæmoglobin obtained from the umbilical-cord blood of four individual babies; all showed two bands of red precipitate after two days. The number of bands of precipitate formed in agar diffusion experiments is an indication of the least number of antigens concerned in the reaction. The distance between the lower boundaries of the two bands shown by cord-blood hæmoglobin differed with different samples of cord blood (see Fig. 1 and Table 1). Antigen 2 was prepared from the cord blood of a premature baby (35 weeks gestation); antigens 3, 4 and 5 were prepared from the cord bloods of babies which were believed to be born at term. Antigen 2 contained 87 per cent of fœtal (alkali-resistant) hæmoglobin estimated by the method of Jonxis[6]; antigen 3 contained 83 per cent of fœtal hæmoglobin. Antigens 4 and 5 had been prepared as carboxyhæmoglobin and Jonxis's method could not be used.

Fig. 2 shows the flocculation times[7] observed in constant antiserum titrations when serial dilutions of the antigens reacted with the antiserum diluted 1 in 25. In contrast with the agar diffusion experiments, none of the titrations disclosed two distinct zones of reaction, but the shapes of the curves, particularly those for antigens 2, 3 and 4, suggest that more than one antigen–antibody system was involved. The optimal ratios[5] of the antiserum determined in the titrations with the five antigens are recorded in Table 1 together with the flocculation times of the optimal mixtures, that is, the first mixture in each titration to show visible particles of precipitate. With the hæmoglobin from the four cord bloods there is a correlation between the flocculation time of the optimal mixture and the distance between the lower boundaries of the two red bands observed in the agar diffusion experiments shown in Fig. 1.

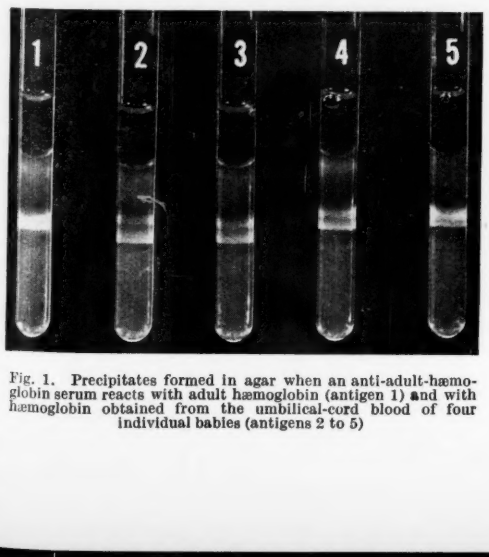

Fig. 1. Precipitates formed in agar when an anti-adult-hæmoglobin serum reacts with adult hæmoglobin (antigen 1) and with hæmoglobin obtained from the umbilical-cord blood of four individual babies (antigens 2 to 5)

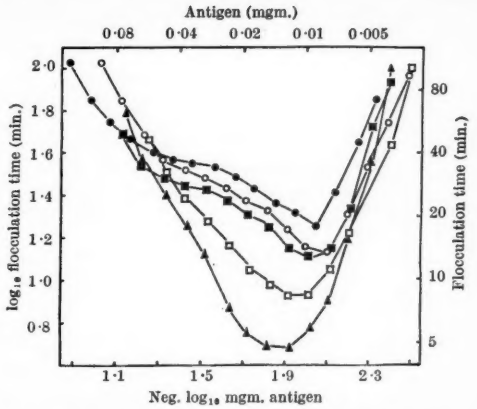

Fig. 2. Constant antiserum titrations of an anti-adult-hæmoglobin serum with adult hæmoglobin (▲, antigen 1) and with hæmoglobin obtained from the umbilical-cord blood of four individual babies (●, antigen 2; ○, antigen 3; ■, antigen 4; □, antigen 5). Antiserum diluted 1 in 25

A red precipitate containing 2·88 mgm. protein was obtained when 1 ml. of the antiserum was mixed with antigen 2 (cord-blood hæmoglobin) in slight antiserum excess of the optimal proportions. When the antiserum was absorbed with an amount of antigen 2 which was approximately three times that in the optimal mixture, a bulky, bright red precipitate formed. After centrifuging, the two bands of oxyhæmoglobin could be easily seen in the supernatant antiserum with a direct-vision spectroscope: the presence of excess antigen was thereby demonstrated. Addition of adult hæmoglobin to this supernatant antiserum gave a red precipitate which contained 1·17 mgm. protein per ml. antiserum. Such distinction of adult and cord-blood hæmoglobin by absorption was not possible with all of twenty antisera examined, which showed considerable differences in the strength of their reactions with adult hæmoglobin after they had been absorbed in antigen excess with cord-blood hæmoglobin. Some absorbed antisera failed to give a ring test with adult hæmoglobin during 2 hr. observation, some reacted feebly and some yielded sufficient precipitate for quantitative determinations. All these twenty antisera reacted rapidly with adult hæmoglobin before absorption.

Aksoy[8] has cited experiments which show a lack of uniformity in the reactions of anti-cord-blood hæmoglobin sera absorbed with adult hæmoglobin. In studies on the reactions of adult and of fœtal hæmoglobin with anti-adult-hæmoglobin sera prepared in chickens, Goodman and Campbell[9] found that individual antisera gave different values for the ratio between the maximum amounts of antibody precipitated by the two antigens. In the present work similar results were obtained with antisera prepared in rabbits.

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FORTHCOMING EVENTS

*(Meeting marked with an asterisk * is open to the public)*

Tuesday, December 29

RADAR AND ELECTRONICS ASSOCIATION (at the Royal Society of Arts, John Adam Street, Adelphi, London, W.C.2), at 7.30 p.m.—Dr. K. R. Sturley: "High Quality Sound Broadcasting".*

Wednesday, December 30

ROYAL SOCIETY OF ARTS (at John Adam Street, Adelphi, London, W.C.2), at 2.30 p.m.—Dr. J. D. Carthy: "Animal Senses and Reactions". (First of two Juvenile Lectures. Further lecture on January 6.)

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, W.C.2), at 3 p.m.—Mr. G. G. Gouriet: "Colour Television". (Christmas Holiday Lecture. To be repeated on Thursday, December 31.)

Friday, January 1

PLASTICS INSTITUTE (in the Lecture Theatre of the Institution of Electrical Engineers, Savoy Place, London, W.C.2), at 2.30 p.m.—Dr. N. A. de Bruyne: "Sticky Plastics". (Sixth Lecture for Young People.)

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

LECTURER OR ASSISTANT LECTURER IN GEOGRAPHY at Makerere College (The University College of East Africa)—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (December 30).

GRADUATE ASSISTANT (with appropriate professional qualifications and/or experience) IN THE COLLEGE LIBRARY—The Principal, Royal Holloway College (University of London), Englefield Green, Surrey (December 31).

RESEARCH FELLOW (with a good degree in electrical engineering, physics or chemistry, and some research experience of vacuum systems) IN THE DEPARTMENT OF ELECTRICAL ENGINEERING—The Registrar, Queen Mary College (University of London), Mile End Road, London, E.1 (December 31).

PROFESSOR OF APPLIED MATHEMATICS—The Secretary and Registrar, University College of North Wales, Bangor, North Wales (January 1).

LECTURER (with a good honours degree in mining engineering and practical experience in mine surveying) IN MINING ENGINEERING AND MINE SURVEYING—The Registrar, The University, Nottingham (January 9).

LECTURERS (with a special interest in servomechanisms, measurements, high voltage or network theory) IN ELECTRICAL ENGINEERING—The Registrar, Manchester College of Science and Technology, Manchester 1 (January 9).

LECTURER OR ASSISTANT LECTURER IN CHEMISTRY at the University of Malaya (Singapore Division)—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (January 15).

CHAIR OF CHEMISTRY—The Registrar, University College of Wales, Aberystwyth (January 16).

VISITING PROFESSOR IN OCEANOGRAPHY at the University of Cape Town, South Africa—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (South Africa, January 31).

HEAD (with high academic qualifications in a branch of soil science or agronomy and considerable research experience and administrative ability) OF THE SOILS DIVISION, Rubber Research Institute of Malaya, Kuala Lumpur, for duties which will embrace the preparation, direction and development of a research programme in soil science nutrition of the rubber tree (*Hevea brasiliensis*) and general assistance in the advisory work of the Soils Division of the Institute—The Secretary, London Advisory Committee for Rubber Research (Ceylon and Malaya), Commonwealth Institute, Imperial Institute Road, London, S.W.7 (March 1).

ASSISTANT (preferably with some knowledge of horticulture and experience in proof correcting and indexing) TO THE EDITOR—The Secretary, The Royal Horticultural Society, Vincent Square, London, S.W.1, marking envelope "Confidential".

ASSISTANT TRIALS OFFICER (preferably with practical experience in crop production), to work at Leeds University Farm, Headley Hall, Yorks—The Secretary, National Institute of Agricultural Botany, Huntingdon Road, Cambridge.

CHEMISTRY MASTER (good sixth form work available for a man of the right qualifications)—The Headmaster, The School, Oundle, Peterborough.

CYTOLOGIST OR CYTOGENETICIST (with experience of chromosomal work), to work on human chromosomal anomalies—The Director of the Medical Research Unit, National Spastics Society, Department of Child Health, Guy's Hospital, London Bridge, London, S.E.1.

EDITOR AND TECHNICAL OFFICER (with experience in editorial work, a sound knowledge of chemistry, and preferably familiarity with one or more foreign languages) to the Society of Dyers and Colourists—The Secretary, The Society of Dyers and Colourists, Dean House, 19 Piccadilly, Bradford, Yorkshire.

LECTURER, ASSISTANT PROFESSOR, and an ASSOCIATE PROFESSOR IN THE DEPARTMENT OF MATHEMATICS—Prof. E. S. Keeping, Mathematics Department, University of Alberta, Edmonton, Canada.

LECTURER (with a good honours degree with teaching and industrial or research experience) IN MATHEMATICS—The Clerk to the Governors, South-East Essex Technical College and School of Art, Longbridge Road, Dagenham, Essex.

ORGANIC CHEMIST, to participate in a biochemical research programme—Dr. Sadler, Courtauld Institute of Biochemistry, Middlesex Hospital Medical School, London, W.1.

READER OR CONSULTANT IN NUCLEAR PHYSICS—The Principal, Bradford Institute of Technology, Bradford.

PHYSICIST (Senior Grade) (with previous experience in hospital physics), to work under the direction of the Principal Physicist in the Radiotherapy Department—The Superintendent, Guy's Hospital, London Bridge, London, S.E.1.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

British Chemicals and Their Manufacture: The Directory of the Association of British Chemical Manufacturers (Incorporated). Pp. 207. (London: Association of British Chemical Manufacturers, 1959.) [3010

Ion Exchange Resins. Fourth edition. Pp. iv+67. (Poole: British Drug Houses, Ltd., 1959.) [3010

Glasshouse Crops Research Institute. Annual Report, 1958. Pp. 94+4 plates. (Rustington, Littlehampton: Glasshouse Crops Research Institute, 1959.) [3010

Biology Staining Schedules for First Year Students. By R. R. Fowell. Sixth edition. Pp. 31. (London: H. K. Lewis and Co., Ltd., 1959.) 3*s*. net. [3010

Bulletin of the British Museum (Natural History). Entomology. Vol. 8, No. 6: Delphacidae from the Lesser Antilles (Homoptera: Fulgoroidea). By R. G. Fennah. Pp. 243–265. 7*s*. Zoology. Vol. 6, No. 1: Taxonomy of the Genus *Columba*. By D. Goodwin. Pp. 1–23. 9*s*. (London: British Museum (Natural History), 1959.) [3010

Rhodes–Livingstone Institute Papers, No. 29: A Preliminary Survey of Luvale Rural Economy. By C. M. N. White. Pp. xii+58. (Manchester: The University Press; New York: Humanities Press, Inc., 1959. Published on behalf of the Rhodes–Livingstone Institute.) 8*s*. 6*d*. net. [3010

Royal Botanic Gardens, Kew. *Kew Bulletin*, General Index to the volumes for the years 1929–1956. Pp. i+300. (London: H.M. Stationery Office, 1959.) 30*s*. net. [3010

Ciba (A.R.L.), Ltd. Technical Notes, No. 202 (October 1959): Hyperbolic Paraboloid Roofs in Industrial Buildings and Schools. Pp. 10. (Duxford: Ciba (A.R.L.), Ltd., 1959.) [3010

Research Defence Society. Conquest Pamphlet No. 9: The Truth About Vivisection: Outstanding Questions posed and answered by the Research Defence Society. Pp. 10. (London: Research Defence Society, 1959.) 6*d*. [3010

Other Countries

FAO/OIE. Animal Health Yearbook, 1959. Pp. 20+vi. (Rome: Food and Agriculture Organization of the United Nations, 1959.) [2210

Tanganyika: Department of Geological Survey. Quarter Degree Sheet 12 S.W.: Oldoinyu Ogol (Serengeti Plain—East). Quarter Degree Sheet 71 S.W.: Chimala. (Dodoma: Department of Geological Survey, 1958.) [2210

United States Department of the Interior: Geological Survey. Bulletin 1052-J: Electrical Properties of Sandstones of the Morrison Formation. By George V. Keller. Pp. iii+307–344+plates 12 and 13. 55 cents. Bulletin 1060-D: Geology and Construction-Material Resources of Nemaha County, Kansas. By Melville R. Mudge, Charles P. Walters and Ralph E. Skoog. Pp. v+179–256+plates 6 and 7. 1 dollar. Bulletin 1074-C: Stratigraphy of the Area between Hernando and Hardee Counties, Florida. By Keith B. Ketner and Lawrence J. McGreevy. Pp. iv+49–124+plates 3–5. 75 cents. Bulletin 1083-A: Periodic Heat Flow in a Stratified Medium, with Application to Permafrost Problems. By Arthur H. Lachenbruch. Pp. iv+36+3 plates. 75 cents. Bulletin 1083-B: Directional Resistivity Measurements in Exploration for Uranium Deposits on the Colorado Plateau. By George V. Keller. Pp. iv+37–72. 20 cents. Bulletin 1083-C: Dissipation of the Temperature Effect of Drilling a Well in Arctic Alaska. By Arthur H. Lachenbruch and Max C. Brewer. Pp. v+73–109. 20 cents. Bulletin 1084-C: Selenium Content of Some Volcanic Rocks from Western United States and Hawaiian Islands. By D. F. Davidson and H. A. Powers. Pp. iii+69–81. 15 cents. Bulletin 1084-E: Distribution of Chemical Elements in the Salt Wash Member of the Morrison Formation, Jo Dandy Area, Montrose County, Colorado. By William L. Newman and Donald P. Elston. Pp. iv+117–150. 20 cents. Bulletin 1106-B: Geophysical Abstracts No. 177, April–June, 1959. By Dorothy B. Vitaliano, S. T. Vesselowsky and others. Pp. iii+129–259. 35 cents. (Washington, D.C.: Government Printing Office, 1959.) [3010

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LETTERS TO THE EDITORS

PHYSICS

Electrical Resistivity Changes observed in a Semiconducting Diamond after Heat Treatment

THE fact that some diamonds are semi-conductors of electricity was first observed by Custers[1]. He designated these as type II*b* diamonds and numerous workers have since reported on the electrical and optical properties of these stones.[2] Recent electrical resistivity measurements on several such diamonds in this Laboratory have revealed that their electrical conductivity can be altered markedly by subjecting them to heat treatment. Initially, these diamonds were heated at 850°C. for 70 hr. in a vacuum of better than 10^{-5} mm. mercury for the required effect but lately the same change has been brought about by heating at 1,400°C. for 3 hr. in an inert atmosphere such as argon.

In this communication we shall describe one particular diamond which is unique in that it consists of a blue and a white portion, both of which are semi-conducting to different degrees and which are separated by a sharp well-defined boundary.

Part of this diamond was sawn off and shaped into a flat rectangular slab of dimensions $1{\cdot}59 \times 5{\cdot}25 \times 7{\cdot}06$ mm. in such a way that the boundary between the blue and white portions roughly bisected the bigger surfaces along their width.

One of these surfaces was then roughened with 8–12 μ diamond powder and small colloidal silver dots were painted on with a camel hair at intervals of about 0·5 mm. so as to bisect the surface along its length and traverse both the blue and white sections of diamond.

Both of the $1{\cdot}59 \times 5{\cdot}25$ mm. ends were also coated with silver to provide end contacts and the diamond was then baked in vacuum for 20 minutes at 400°C. to ensure good bonding between the diamond and silver. Prepared in this way the contact resistance was found to be negligible.

On passing a known current through the diamond by way of the end contacts the resulting potential differences between the silver dots could be measured accurately by means of a sensitive vacuum voltmeter. Distances between the centres of the silver dots were measured with a travelling microscope. Let i be the current in amp. flowing through the diamond and v the potential difference in volts between any two silver dots distance x cm. apart.

Then $v/i = \rho x/A$, where A is the area of an end contact in cm.^{2} and ρ the electrical resistivity.

Therefore $\rho = \frac{dy}{dx} \cdot \frac{A}{i}$.

In Figs. 1 and 2 the potential difference along the diamond is plotted as a function of the distance from one edge before as well as after heat treatment. It is obvious that there is a big difference between dy/dx for the white and the blue portions of diamond before heating which becomes not nearly so marked after heat treatment. dy/dx and, therefore ρ, becomes considerably smaller after heating and this is specially noticeable with the white portion. A and i being known, resistivities are evaluated in Table I.

TABLE 1

	ρ of blue portion	ρ of white portion
Unheated	97·8 ohm cm.	20,500 ohm cm.
Heated	56·5 ohm cm.	313 ohm cm.

Semi-conducting diamonds are *p*-type, that is, they are dependent upon acceptor centres for conduction.

Let us assume that the acceptor concentration is uniform throughout the diamond whether it is white or blue. This assumption is reasonable as acceptor centres are thought to be substitutional group III impurity atoms in the lattice and would require an extremely high threshold energy to move them. This energy is higher than the thermal energy provided at 850°C. or 1,400°C. In diamond donor centres also exist. If these approach, numerically, the number of acceptors the conductivity decreases until, when the number of donors equals the number of acceptors, the diamond becomes an insulator.

Thus in the white portion of the diamond with a low conductivity there must be more donors present if the acceptor level is constant. This would also account for the lower intensity of blue colour since the blue colour is due to absorption in the near infra-red by the 0·35 eV. acceptor-level. With the

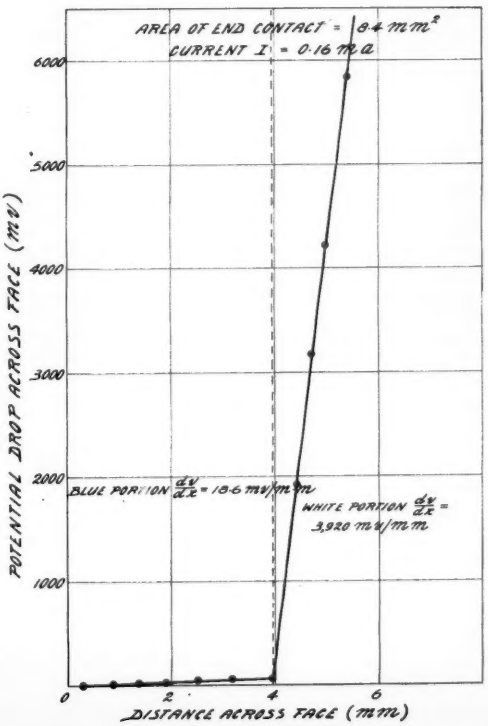

Fig. 1. Unheated diamond

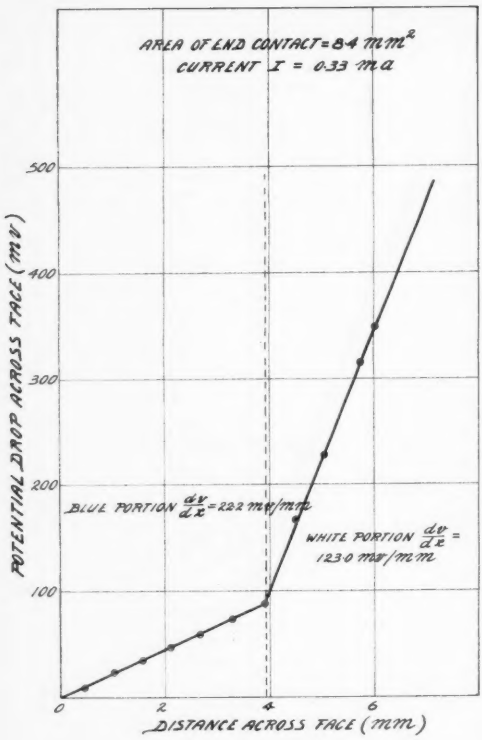

Fig. II. Heated diamonds

nullifying of this level by increase in donor concentration the diamond becomes white.

From work by Wedepohl[3] it is known that displaced atoms in the diamond lattice give rise to donor centres. These displaced atoms could have been formed in the original crystallization or, in this case, considering the well-defined boundary, by being in the vicinity of some radio-active decay process.

The reduction in donor concentration in both the blue and the white portions on heating appears to be consistent with a bimolecular annealing process, that is, an interstitial carbon atom moving to a vacant site in the lattice with consequent decrease in donor centres and a corresponding decrease in resistivity.

For bimolecular annealing the rate at any stage is proportional to N^2 where N is the number of defects. Thus, the white portion with more donor centres will show a greater difference in resistivity than the blue portion on heating for the same length of time.

The authors' thanks are due to Messrs. Industrial Distributors (1946) Ltd. for permission to publish this work and also to Dr. J. F. H. Custers, director of research, for his interest.

R. E. Mutch
F. A. Raal

Diamond Research Laboratory,
Johannesburg.
Oct. 28.

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Vibrational Relaxation and Dissociation of Carbon Dioxide behind Shock Waves

We have recently[1,2] adapted the sodium-line reversal method of measuring temperature to make time-resolved studies behind shock waves, and have shown that in some cases departures from the calculated equilibrium temperature can be interpreted in terms of vibrational or dissociative relaxation processes. The sodium excitation temperature follows the effective vibrational temperature of the molecules, so that, if there is a lag in the partition of vibrational energy, the measured temperature is initially too low; and, if the vibration lag is short but there is a delay in attainment of dissociative equilibrium, the initial observed temperature is too high.

For shocks of Mach number between 8 and 10 through carbon dioxide, we find evidence of both vibrational relaxation and delayed dissociation. The sodium excitation temperature close to the shock front is quite low, but rises rapidly to a value far in excess of the equilibrium temperature, but close to the calculated temperature for no dissociation, and then decays slowly towards the equilibrium value; in our experiments, the time of flow is inadequate for complete equilibrium to be attained. Fig. 1 shows the variation of sodium excitation temperature with time behind a shock at Mach 9·41; this is derived from analysis of a double-beam record; the calculated equilibrium temperature at this Mach number is 2,400°K. and for no dissociation 2,740°K. (ref. 3); the time scale is shown in laboratory co-ordinates; in shock-fixed co-ordinates it would be longer by the density ratio, that is, by a factor of about 12; the pressure behind the shock wave is calculated to be 0·37 atm. It will be seen that the observed temperature rises from a very low initial value almost to that for no dissociation (2,740°K.). and then falls slowly, reaching about 2,600°K. at the contact surface.

Assuming that equilibrium is attained in an exponential manner, and that the dissociation proceeds at a much slower rate than vibrational relaxation, we have calculated the relaxation times for vibration and dissociation from several records. The times are defined as those required for the departure from equilibrium to decay to $1/e$ of its previous value and are reduced to standard pressure, assuming the rates are proportional to pressure. For carbon dioxide of commercial purity, we obtain vibrational relaxation times of 6 μsec. at 2,720°K., 32 μsec. at 2,550° and 54 μsec. at 2,520°; these temperatures are the observed peak temperatures, and exceed the equilibrium values. For dissociation, the relaxation time from the record shown in Fig. 1 is about 350 μsec., this being measured over an average temperature of about 2,650°K., whereas the equilibrium value is only 2,420°. Another similar record at about the same temperature gives 600 μsec. for the dissociation relaxation time.

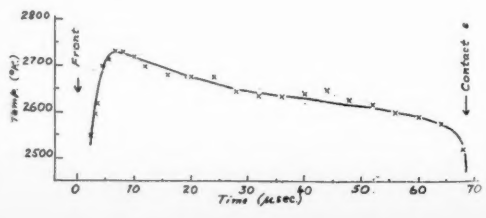

Fig. 1

Both vibration and dissociation rates are sensitive to the presence of impurities. Addition of 2 per cent water vapour reduced the dissociation time to 100 μsec. for a shock with equilibrium temperature 2,445°K. The vibrational relaxation time was measured as only 23 μsec. at 2,270°, and was too short to record at the higher temperatures for which results were obtained for pure carbon dioxide.

Carbon dioxide possesses three vibrational modes; the symmetrical mode ν_1 has a frequency of 1,388 cm.$^{-1}$, the transverse mode ν_2 one of 667 cm.$^{-1}$ and the asymmetric mode ν_3 one of 2,349 cm.$^{-1}$. Ultrasonic dispersion is dominated by relaxation of the lowest frequency mode ν_2 and shows a relaxation time which at high temperature would be much too short to observe under our conditions. ν_1 makes a smaller contribution to the specific heat at low temperature and does not show a well-defined dispersion frequency; it is usually assumed that inter-conversion of energy from one mode to another on collision would lead to the two frequencies having the same relaxation time, although there is at least one report[4] of a shelf on the sound dispersion curve which might be identified with separate relaxation of ν_1. The asymmetric mode ν_3 has too high a frequency to make an appreciable contribution to the specific heat in the temperature range of ultra-sonic studies. It has seemed uncertain whether a separate relaxation time should exist for this high-frequency mode or whether interconversion of energy between the three vibrational forms should lead to a single relaxation time. For sulphur dioxide, there is evidence for two separate relaxation times[5]. The present results appear to indicate that carbon dioxide has a separate relaxation time for the asymmetric mode which is of the same order as the vibrational relaxation times of nitrogen and carbon monoxide, which have comparable vibrational frequencies (2,360 cm.$^{-1}$ and 2,170 cm.$^{-1}$ respectively). This separate relaxation of ν_3 seems reasonable, since it is difficult to visualize an efficient process of transfer of vibrational energy between the asymmetric and valence modes.

The relatively long time for the dissociation of carbon dioxide and the influence of water on this is of interest for the time to attain equilibrium following combustion. There is much evidence for delayed burning of carbon monoxide and for the action of water vapour as catalyst.

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Phase Transitions in Interlamellar Films

A previous communication[1] has described the interlamellar sorption of straight chain amines by graphic acid. Attention was then directed to a break in the curve of spacing against chain-length, occurring between 9 and 10 C-atoms per molecule.

This we now believe to be due to the change of the sorbed film from the liquid to the solid state. If this assumption is accepted, it follows that the sorbed films solidify more readily than the pure substances, for which the liquid-solid transition occurs between 11 and 12 C-atoms.

Evidence for the assumption is provided by observation, on the X-ray diagram, of a diffraction in the region of 4·3A. This consists of a broad band on the diagram of the complexes with the lower amines, but it sharpens to a sharp line concurrently with the spacing shift. This band or line is presumably due to the spacing between neighbouring chains.

Further evidence is provided by X-ray diagrams of sorption complexes with mixtures of two amines, that is, hexylamine and decylamine. These give intermediate spacings which, when plotted against the proportion of higher amines in the mixture (Fig. 1) give a straight line. The point for pure

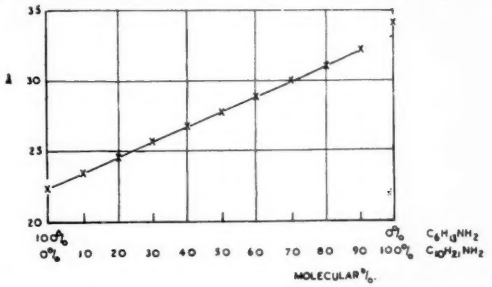

Fig. 1. Interlayer spacings of graphitic acid complexes with mixtures of hexylamine and decylamine, plotted against proportion of higher amine

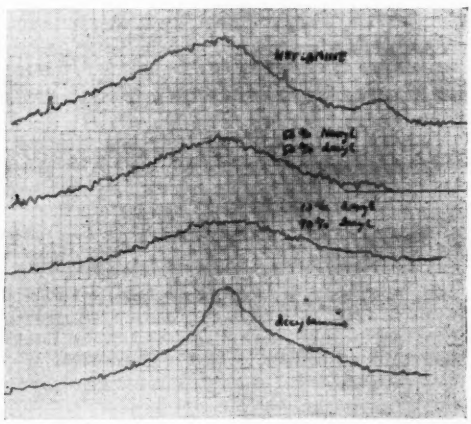

Fig. 2. Microphotometer curves of the diffraction maximum in the region of 4·3A. for certain of the above mixtures in Fig. 1

decylamine, however, lies off the sharper line. Here again, the nature of the diffraction at 4·3A. changes concurrently with the break in the graph (Fig. 2). We have made several similar observations.

Detailed observation suggests that the liquid-solid change in these sorbed films is not sharp, but that the 4·3A. line sharpens over a range of values following the break.

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GEOPHYSICS

Palæomagnetism of Some Dolerite Intrusions from the Theron Mountains and Whichaway Nunataks, Antarctica

As a part of the scientific activity of the Commonwealth Trans-Antarctic Expedition a collection of eight oriented samples was made by Stephenson from dolerite sills and dykes in the region of the Theron Mountains, Shackleton Range and Whichaway Nunataks. The seven intrusions which have been sampled bear petrological affinities with the Karroo Dolerites of South Africa and with the dolerite sills of Tasmania, and are thought to be of comparable age. They intrude flat-lying sediments and show no evidence of post-formational tilting.

The directions of remanent magnetization have been measured by Blundell and are shown in Fig. 1. These are arranged in two groups, normal and reversed, which are 180° apart. The two groups have been combined by reversing the polarity of those with downward dips to provide a mean direction of magnetization with azimuth 64° E. of N. and dip 68° up. The circle of 95 per cent confidence, applying Fisher's statistics[1], has a radius of 12 deg.

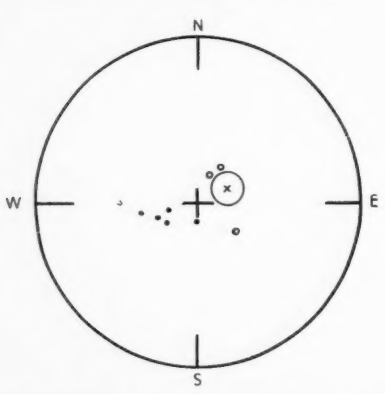

Fig. 1. Stereogram of directions of magnetization of Antarctic Dolerite intrusions. ○, dip up ; ●, dip down ; ×, mean direction with circle of 95 per cent confidence

Storage tests similar to those already described[2] were applied during the measurements of magnetic direction and indicated a stable magnetization. It is also evident from Fig. 1 that there is a high degree of stability, for the normal and reversed groups have remained 180° apart and at a substantial angle to the present field direction. The samples are unaltered chemically and there is no evidence of the development of any secondary magnetization, so it may be presumed that the mean direction of magnetization is a true record of the field direction when the rocks were intruded. Since seven intrusions from a wide area were sampled, the effects of local variations in the geomagnetic field and of secular variation are unlikely to be significant.

Over a period of 1,000 years or more, the geomagnetic field appears on average to resemble that of a geocentric axial dipole. On this basis the position of the (south) pole relative to the Theron Mountains when the sills were intruded was at lat. 54° S., long. 136° W. with an oval of 95 per cent confidence of 16° radius. This pole position may be compared with those similarly derived for the Karroo Dolerites

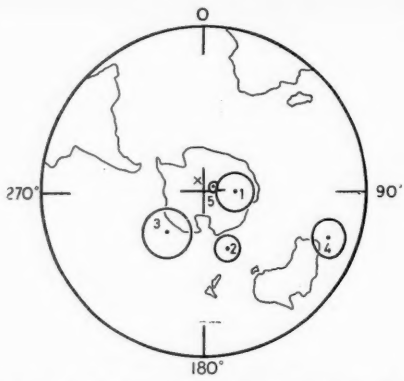

Fig. 2. Jurassic Poles for Africa (1), Australia (2), Antarctica (3), India (4) and South America (5), with ovals of 95 per cent confidence. ×, sampling area

of South Africa[3], the Tasmanian Dolerites[4], the Rahajmahal Traps of India[5] and the Serra Geral lavas of Brazil[6], shown in Fig. 2. The age correlation between these rocks is not good, but it may be stated with some confidence that they are all Jurassic, and are the same age to within 30 million years. The difference between the Antarctic and Indian pole positions is more than 100°, and though this could be explained in terms of Gold's mechanism[7] for polar wandering, it seems unlikely in view of the relatively small variation in pole positions from the Jurassic to the present day relative to a single continent. The scatter in pole positions shown in Fig. 2 is more indicative of relative movement of the continents, and it is interesting to note that these results are not inconsistent with earlier reconstructions put forward on independent evidence by proponents of the continental drift hypothesis.

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Palæomagnetism in Some Norwegian Sparagmites and the Late Pre-Cambrian Ice Age

Pre-Pleistocene glacial deposits have been claimed from many ages and latitudes. Palæomagnetic studies of the Permian tillites in Australia[1] suggest that they were near the geomagnetic, and probably also the geographical pole. The late Pre-Cambrian ('Eo-Cambrian', Varangian, Sinian, etc.) tillites are of special interest for several reasons :

(1) Widespread occurrences have been claimed[2] and many are well established, for example, in Australia, China, Greenland, Norway and Spitsbergen.

(2) Their stratigraphical position suggests that all are referable to one major glacial period (with, however, interglacial or interstadial intervals). It is therefore a key horizon in international correlation below the fossiliferous Lower Cambrian.

(3) The boulder beds usually suggest deposition from floating ice in an apparently continuous marine succession.

(4) Palæomagnetic determinations of Palæozoic and late Pre-Cambrian rocks from Britain and America[3] give pole positions which suggest that the Norwegian and Arctic tillites were deposited not far from the geomagnetic equator. The successions in which they occur show facies consistent with a near equatorial position.

In the course of investigations of the magnetism of some Arctic rocks, collections of tillites and associated horizons were made by us on Dr. Lauge Koch's East Greenland Expedition in 1957, and from Spitsbergen and southern Norway in 1958. This communication gives the results of the smallest of these (29 specimens collected from the sparagmites on Lake Mjösen in southern Norway by W.B.H.) and discusses them in the light of work on the Greenland and Spitsbergen material.

Holtedahl first suggested a glacial origin for some of the Moelven conglomerates[4] and the succession sampled was first mapped by Goldschmidt[5]. Table 1 gives the succession as summarized by Holtedahl[6] and the stratigraphical position of the samples collected. We are indebted to Dr. T. Strand of Oslo for advice as to the best localities. The rocks were

TABLE 1

	Horizon	No. of sites	No. of specimens	No. of specimens giving significant results
9	Ringsaker Quartzite	3	9	3
8	Vardal Sparagmite	2	5	3
7	Ekre Shale			
6	Moelv Conglomerate and Tillite	3	6	4
5	Moelv Red Sparagmites	1	1	1
4	Biri Limestone			
3	Biri Conglomerates	2	5	—
2	Bröttum Limestones and Shales			
1	Bröttum Grey Sparagmites	1	1	—

generally coarse, and would not have been selected for palæomagnetic work but for their critical stratigraphical position. Samples were orientated in the field by rock compass[7]. In the laboratory the rocks were re-oriented, cored, and measured on the astatic magnetometer, similar in construction to that described by Collinson *et al.*[8], which is housed at the Department of Geodesy and Geophysics, Cambridge. For these facilities we wish to thank Mr. B. C. Browne and Dr. J. C. Belshé. From each rock three samples were measured, the vectors combined by meaning the direction cosines and a statistical estimate of the dispersion made using the method proposed by Fisher[9]. These mean vectors were then combined to give the pole position[10], the main computations being carried out on the Cambridge digital computor *Edsac*.

Of the twenty-nine rocks collected, eighteen were too weakly magnetized and too inhomogeneous to give significant results. The remaining eleven specimens gave the mean vectors listed in Table 2 and plotted on the equal area stereonet in Fig. 1 after correction for observed geological dip. The vectors fall into two groups.

TABLE 2

Specimen No.	Horizon No.	Locality	Field Position D	Field Position I	Dip Corrected D	Dip Corrected I	α	κ	Intensity (10^{-3} G./c.c.)
MN 07·3	9	Coast N. of Evjeviken	334	29	377	−8	20	38	1·4
MN 02·3	9	Coast Skotta	172	−28	171	24	14	83	13·7
MN 02·4	9	Coast Skotta	186	−8	190	33	32	16	6·2
MN 06·2	8	N.E. of Moelven	042	72	008	7	4	896	3·8
MN 09·1	8	Coast S. of Smedstad-viken	198	10	205	42	16	57	1·6
MN 09·2	8	,, ,,	211	13	221	43	17	51	0·4
MN 03·1	6	Coast N. of Tyvehl	151	27	124	70	18	49	1·1
MN 04·1	6	Coast S. of Tyvehl	120	20	105	23	12	109	3·3
MN 04·2	6	,, ,,	237	23	257	47	4	993	1·0
MN 04·3	6	,, ,,	275	53	324	28	11	122	10·3
MN 12·1	1	E. of Bergsodden	217	−7	215	−6	9	194	12·1

The following abbreviations are used: *D* declination in degrees measured from true north; *I* inclination to the horizontal in degrees, positive downwards, negative upwards; α semi-angle of the cone of confidence drawn at 95% probability level; κ estimate of precision.

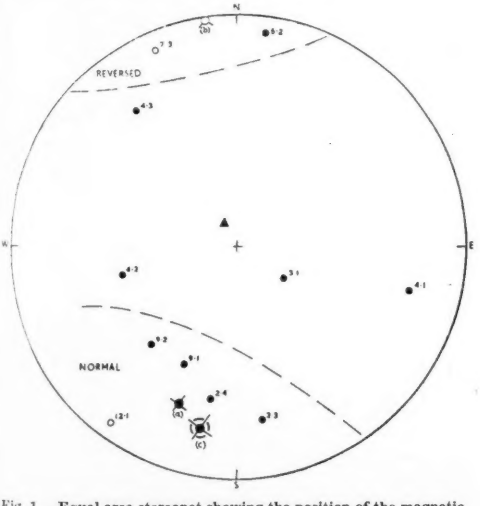

Fig. 1. Equal area stereonet showing the position of the magnetic vectors for eleven rocks from the Norwegian tillites together with the mean vectors derived from the normal, reversed, and the combined normal and reversed groups. ●, Vector plotting in lower hemisphere; ○, vector plotting in upper hemisphere; *a*, mean vector normal group, lower hemisphere; *b*, mean vector reversed group, upper hemisphere; *c*, mean vector of combined normal and reversed groups in lower hemisphere; ▲, direction of present magnetic field

The first group consists of four rocks collected at two sites with mean vectors which show a considerable scatter but with a mean direction:

$$D\ 352°,\ I\ 87°,\ \alpha 89°,\ \kappa 2$$

which lies very close to the direction of the present magnetic field in the area. We think these rocks have been partially magnetized in the direction of the present field.

The second group consists of five vectors lying in the south-west quadrant and two vectors which are nearly opposite in position, but which if reversed would fall close to the first five vectors. We think that this was caused by the Earth's magnetic field reversing its polarity at least twice during the time span covered by the rocks collected. Combining the five normal vectors with the two reversed vectors, after these have been re-reversed, gives a mean direction:

$$D\ 191°,\ I\ 21°,\ \alpha 24°,\ \kappa 7.$$

This gives a position for the corresponding magnetic pole at:

Lat. 18°N., Long. 179°E.

Despite the relative uncertainty due to the small number of rocks and their generally weak magnetization, the resulting pole position is close to that for the Cambrian of Great Britain[11] and also to tillites from Greenland collected by us (Fig. 2), and intermediate to the pole positions for rocks of Silurian and Late Pre-Cambrian ages from the United States[3]. In all these cases, and also for the tillites of comparable age collected from Spitsbergen and at present

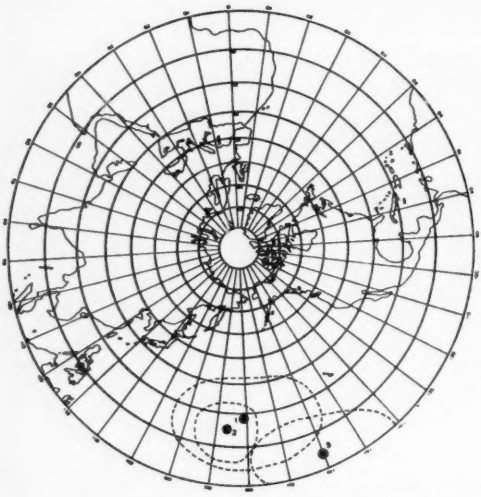

Fig. 2. Polar projection showing positions of magnetic poles derived from 1, Norwegian tillites; 2, Caerbwdy Sandstones Wales; 3, Greenland tillites. The broken lines show the ovals of confidence drawn at the 95 per cent level.

being investigated, the collecting areas appear to have lain in equatorial regions not far removed from the earth's geomagnetic equator. If it be assumed that the Earth's magnetic pole, when averaged over long periods, has been close to the geographical pole, the palæomagnetic evidence suggests that at the close of Pre-Cambrian time Scandinavia, Spitsbergen and Greenland were equatorial rather than polar regions.

Pole positions for the tillite horizons are therefore consistent with the palæomagnetic picture already obtained, at least as regards latitude, and it is probable that marine tillites were formed near the equator in late Pre-Cambrian time. This evidence, together with the world-wide distribution of supposed tillites of the same age, argues an ice age far more severe than any later ice age. The thickness of many tillite deposits, together with some evidence from Greenland rocks of appreciable change in the geomagnetic field during the tillite period, suggest a duration many times that of the Quaternary Ice Age to date.

Such an event may have profound significance in the understanding of late Pre-Cambrian–Cambrian stratigraphy. In particular, the sudden widespread appearance of highly organized life in Lower Cambrian times may in part be the result of a general relaxation of prolonged severe glacial control both of faunas and sea level as already suggested[12]. The physiographical and astronomical implications of such a glaciation are also worthy of note.

The extension downwards of an internationally acceptable stratigraphical system beneath the Cambrian is now much discussed because of the similarity of many transitional successions throughout the world. For this the glacial horizons, already recognized as providing a stratigraphical datum, may yield a succession capable of correlation and worthy of status as an infra-Cambrian system (?Varegian[13]). The establishment of new systems is premature without adequate correlation and a recent conference[14] concluded that radioactive dating might provide such a basis. Our work suggests that palæoclimatic and palæomagnetic correlation together promise a more immediate means of correlation within these late Pre-Cambrian successions.

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METALLURGY

X-Ray Diffraction Pattern of Carbide in Low-Carbon Iron-Silicon Alloys

A CARBIDE which has a needle-like appearance on a polished and etched cross-section is a common occurrence in hot-rolled 3·25 per cent silicon iron. It is metastable with respect to cementite and can be formed by a variety of heat treatments[1].

An electron-transmission diffraction pattern was obtained from a disk (carbide single crystal) separated from a commercial 3·25 per cent silicon steel containing 0·03 per cent carbon[1]. This pattern could be indexed on the basis of a cubic or tetragonal structure with $a_0 = 6{\cdot}06$ A. It was concluded, however, that these structures were unlikely because of a large number of omissions in the pattern, and that the crystal structure might be complex with a large unit cell. It was obvious that the carbide was neither cementite nor epsilon carbide ($Fe_{2\cdot4}C$).

A number of attempts were made to obtain a reproducible X-ray diffraction pattern from hot-rolled 3·25 per cent silicon steel, containing 0·03 per cent carbon or less. All these failed, presumably because the amount of carbide present was insufficient. To obtain a reproducible pattern, it was necessary to increase the carbon to 0·06 per cent, dissolve it in ferrite at a high temperature, quench, then age to develop the precipitate.

A 2-lb. vacuum melt containing 0·062 per cent carbon and 3·51 per cent silicon was prepared. To dissolve all the carbide in ferrite, samples of this alloy were heated to 2,540°F. (1,395°C.) for ½ hr. in evacuated silica capsules. After brine quenching, the samples were aged at 300°F. (150°C.), 600°F. (315°C.) or 800°F. (425°C.) for various periods. These treatments produced a large quantity of oriented precipitate (Fig. 1). After ageing, blanks from each sample were machined to small cylinders, 1 mm. diameter × 5 mm. long, then etched in nitric acid to 0·5 mm. diameter. Adjacent pieces were prepared for metallographic examination. The specimens for X-ray examination were given a final etch in picral, nital or 2 per cent iodine-ethyl alcohol to put the precipitate at the surface in relief, then washed in methanol and dried. These specimens were mounted in a 114 mm. Debye-Scherrer camera and exposed to iron-filtered cobalt radiation at 40 kV. and 10 m.amp. for various periods. Since several grains in each sample were irradiated, a

Table 1. X-Ray Diffraction Data Specimen Aged 800°F.—20 Min. Iron Filtered Co Kα Radiation

1	2	3	4	5	6
'd'	I	$\sin^2\theta$ (obs.)	$\sin^2\theta$ (calc.)	difference between calc. and obs.	*hkl*
7·76	M	0·0133	0·0137	+0·0004	011
7·63	VW	0·0137	0·0143	+0·0005	101
7·24	VS	0·0153	0·0154	−0·0001	002
4·48	W	0·0398	0·0398	0·0000	020
4·41	W	0·0412	0·0418	−0·0006	200
4·31	W	0·0430	0·0437	−0·0007	021
4·18	W	0·0459	0·0457	+0·0002	201
3·81	W	0·0551	0·0553	−0·0002	022
3·71	VW	0·0580	0·0573	+0·0007	202
3·60	VS	0·0618	0·0618	0·0000	004
3·23	VW	0·0769	0·0766	+0·0003	203
3·08	M	0·0847	0·0855	−0·0008	221
2·87	VW	0·0973	0·0979		301
			0·0966		005
2·57	W	0·1211	0·1195		312
2·54	VW	0·1238	0·1243		033
2·51	W	0·1269	0·1288		303
2·395	M	0·1397	0·1388		313
			0·1391		006
2·351	W	0·1448	0·1434		224
2·307	VW	0·1504	0·1496		106
			0·1514		034
2·075	VW	0·1860	0·1861		035
2·031*	M	0·1938	0·1926		043
1·907	VW	0·2204	0·2184		333
			0·2207		226
1·896	VW	0·2229	0·2210		044
			0·2225		422
1·796	W	0·2485	0·2488		050
			0·2472		008
1·728	VVW	0·2684	0·2687		342
			0·2688		424
1·668	W	0·2879	0·2880		343
1·627	VW	0·3089	0·3088		146
1·584	W	0·3192	0·3186		434
1·541	W	0·3374	0·3368		038
1·495	W	0·3582	0·3587		147
			0·3582		080
1·406	W	0·4055	0·4047		354
			0·4064		048

Suggested orthorhombic structure
$a_0 = 8{\cdot}8$ A., $b_0 = 9{\cdot}0$ A., $c_0 = 14{\cdot}4$ A.

* Could be Fe (110) Line.

'powder' pattern was produced by transmission despite the oriented nature of the precipitate in each grain.

Specimens aged at 300°F. (150°C.) and 800°F. (425°C.) gave similar and reproducible diffraction patterns (Table 1). In three attempts, an iron-3·5 per cent silicon alloy, containing only 0·0025 per cent carbon, similarly treated, with no visible precipitate present, gave no diffraction pattern other than the lines from the ferrite matrix.

To guard against the possibility that the diffraction pattern originated in surface contamination from the final etch, the etching was altered as previously described, but the diffraction pattern did not change. A total of 6 similar patterns were obtained from specimens aged at 300°F. (150°C.) and 10 from specimens aged at 800°F. (425°C.). In three attempts, no patterns were obtained from specimens aged at 600°F. (315°C.), although a large amount of precipitate was present. The reason for this lack of diffraction is not known.

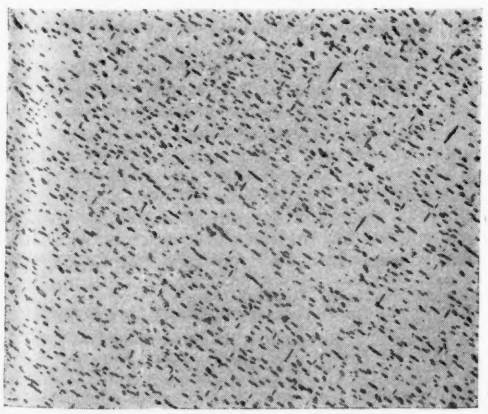

Fig. 1. Precipitate found in iron −3·51 per cent silicon −0·06 per cent carbon alloy aged at 800°F. (425°C.) for 20 min. Picral etch (×600)

The diffraction patterns obtained cannot be reconciled with the previous electron diffraction evidence[1], but they do support the conclusion that the carbide is neither cementite nor epsilon. The patterns also do not agree with the patterns[4] obtained for various iron-silicon carbides prepared by Marles[2], Hurst and Riley[3] and Owen and Street[4].

The determination from powder patterns of a structure having low symmetry is very difficult, and the results should be regarded with caution. The assignment of an orthorhombic structure to this metastable 'needle' carbide, with $a_0 = 8{\cdot}8$ A., $b_0 = 9{\cdot}0$ A., $c_0 = 14{\cdot}4$ A. must be regarded as tentative. The possibility of obtaining a single crystal of the carbide for X-ray diffraction studies seems remote.

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CRYSTALLOGRAPHY

Crystal Structure of L-Serine Phosphate

A number of very accurate crystal-structure determinations of amino-acids have been reported during the past decade, and these have provided a valuable basis for theories regarding the configurations of polypeptide chains in proteins. Serine and threonine contain the grouping C_β-OH and can presumably occur in proteins with this group esterified: the phosphoric acid ester of serine has in fact been obtained as a product of the acid hydrolysis of casein[1,2]. In view of this, and since, moreover, accurate dimensions for both the serine[3] and threonine[4] molecules are available, an examination of the crystal structure of L-serine phosphate was undertaken by us and a lengthy refinement using three-dimensional intensity data has now reached a stage at which accurate atomic co-ordinates are available.

L-Serine phosphate, which, as the result of the following analysis, may be written $^-HO_3POCH_2CH(NH_3^+)COOH$, crystallizes in the orthorhombic system with lattice parameters $a = 7{\cdot}79 \pm 0{\cdot}01$, $b = 10{\cdot}24 \pm 0{\cdot}04$, $c = 9{\cdot}09 \pm 0{\cdot}02$ A. There are four molecules in the unit cell, $D_x = 1{\cdot}692$, $D_m = 1{\cdot}707$, and the space group is $P2_12_12_1$.

As prolonged attempts to elucidate the crystal structure in projection were unsuccessful, resort was made to the three-dimensional Patterson function, kindly calculated for us by Prof. R. Pepinsky on *XRAC*, and this was interpreted by a superposition method[5]. The approximate atomic co-ordinates so deduced were improved initially by two-dimensional methods, and latterly by least-squares refinement using the full three-dimensional data. After seven cycles of such refinement, including allowance for anisotropic atomic vibrations, the average discrepancy over the 935 (*hkl*) reflexions is now 103 per cent.

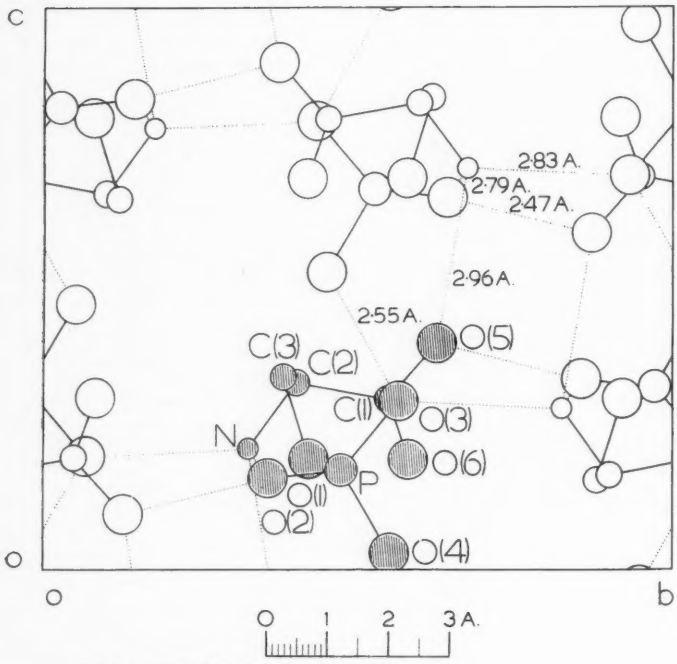


Fig. 1. L-serine phosphate. *a*-axis projection

A view of the crystal structure projected down the *a*-axis is shown in Fig. 1 with the hydrogen-bonded contacts between molecules indicated. Distances between directly bonded atoms are listed in Table 1.

Table 1. Bond-lengths in L-serine Phosphate

	Distance (A.)		Distance (A.)
C(1)–O(6)	1·201	C(3)–O(1)	1·466
C(1)–O(5)	1·321	P–O(1)	1·608
C(1)–C(2)	1·541	P–O(4)	1·560
C(2)–N	1·468	P–O(2)	1·517
C(2)–C(3)	1·526	P–O(3)	1·497

The carboxyl group, the nitrogen atom and the carbon atom C(2) are accurately coplanar, the maximum displacement from the mean plane being 0·009 A. (atom C(2)). The chain C(2)-C(3)-O(1)-P-O(4) is roughly planar and is inclined at approximately right angles to the plane of the carboxyl group and nitrogen atom. The configurations around the C(2)-N and C(2)-C(3) bonds are staggered (ethane-like) with atom O(1) occupying what is apparently the least favourable of the three possible positions, close to both the carboxyl and amino-groups, as shown in Fig. 2. This configuration is found also in serine but not in threonine.

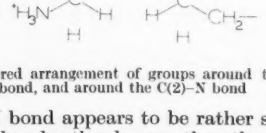
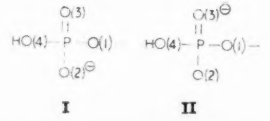


Fig. 2. Staggered arrangement of groups around the C(2)–C(3) bond, and around the C(2)–N bond

The C(2)-N bond appears to be rather shorter, and the C(3)-O(1) bond rather longer, than the corresponding bonds in serine and threonine for which d(C-N) = 1·491, 1·490 A., respectively; d(C-O) = 1·425, 1·424 A. respectively. In dibenzyl phosphate[6], however, C-O lengths of 1·464 and 1·465 A. are found. The dimensions of the C-O bonds in the carboxyl group, 1·201 and 1·321 A., clearly indicate that this group is not ionized and that the 'zwitterion' structure is formed by ionization of the phosphoric acid group.

In dibenzyl phosphate a value of 1·469 A. is reported for the P=O bond. Both the P—O(2) and P—O(3) distances of 1·517 and 1·497 A. in serine phosphate are distinctly longer than this but shorter than the P—O(4) distance. At 1·560 A. this distance is in good agreement with the value of 1·545 A. reported for the P—OH bond in dibenzyl phosphate. Taken in conjunction with the arrangement of hydrogen-bonded contacts, all this suggests strongly that the negative charge on the phosphoric acid group in serine phosphate is shared between atoms O(2) and O(3), the true structure being a 'resonance hybrid' of the two forms I and II.

The P—O(1) bond at 1·608 A. is distinctly longer than the corresponding bonds in dibenzyl phosphate, namely 1·545 and 1·566 A., and in better agreement with the value of 1·62 A. reported for P-OP bonds in P_4O_{10} (ref. 7) and $(NH_4)_4P_4O_{12}$ (ref. 8).



I II

The nitrogen atom forms three hydrogen bonds with d(N-H · · · O) = 2·79, 2·83 and 2·96 A., values which are normal for this type of atomic separation. One of the two oxygen—oxygen hydrogen-bonded separations is notably short. This involves the hydroxyl oxygen of the carboxyl group and a negatively charged oxygen of the phosphate group with d(O-H · · · $O^{1/2-}$) = 2·47 A. The other hydrogen bond involves the hydroxyl oxygen of the phosphate group and a negatively charged oxygen of another phosphate group with d(O-H · · · $O^{1/2-}$) = 2·55 A.

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CHEMISTRY

An Attempt at Calculating the Taft's Constants σ*

In our study of the influence of structure on the reactivity and properties of non-aromatic compounds in homologous series we have investigated the possibility of calculating Taft's[1] polar substituent constants σ*. It is well known that there exists a connexion between the electron density on the carbon atom (C_f) to which the functional group is linked and the value of σ*.

We obtained a very close correlation between these two quantities when the electron densities were calculated by means of a very simple method recently proposed by Del Re[2]. The dependence is linear. The data for saturated, non-substituted derivatives lie on one straight line, while the data for substituted alkyls are situated on another (Fig. 1), consequently

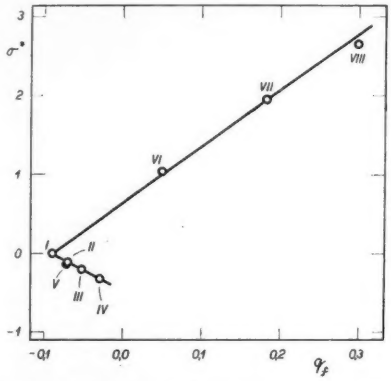

Fig. 1. The dependence of Taft's σ* constants on the electron density on the carbon atom C_f: I, methyl; II, ethyl; III, iso-propyl; IV, *tert* butyl; V, *n*-propyl; VI, chloromethyl; VII, dichloromethyl; VIII, trichloromethyl

the relation between the electron densities according to Del Re and Taft's σ* constants is defined by:

$$\sigma^* = a + bq_f \qquad (1)$$

where a and b are numerical constants, which are negative for the first group of substituents (alkyls) and positive for the second group (Table 1) and q_f is the electron density on C_f. This relation is formally the same as the one found by us[4] for the connexion between the basicity or the position of the absorption maximum of N-alkylthioureas and the constants σ*.

By means of equation (1) we can, on one hand, predict the values of σ* for substituents for which this value has not been determined from experimental data and, on the other hand, verify or propose the values of the empirical parameters occurring in the above mentioned method[2]. Further details will be published later.

I am indebted to Dr. J. Koutecký for valuable discussions and suggestions.

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Table 1. Published and calculated values of σ*

Alkyl	Methyl	Ethyl	*iso* Propyl	*tert* Butyl	*n*-Propyl	Chloro-methyl	Dichloro-methyl	Trichloro-methyl
Refs. 1 and 3	0·000	−0·100	−0·200	−0·320	−0·115	1·050	1·940	(2·65)
Calculated by equation 1†	−0·008	−0·109	−0·204	−0·326	−0·101	0·996	1·931	2·752

† Values of numerical constants a and b (equation 1): $a = -0{\cdot}485$ and $b = -5{\cdot}30$ for saturated, nonsubstituted substituents, and $a = 0{\cdot}642$ and $b = 7{\cdot}08$ for the second group of substituents.

Estimation of Chain Fracture and Cross-Linking of Rubber by High-energy Radiation

Recently the solubility behaviour of irradiated polymers has been analysed theoretically by Charlesby and Pinner[1]. The soluble fraction (s) of a cross-linked network of macromolecular material, initially of randomly distributed molecular weights, can be written:

$$s + s^{1/2} = \frac{p}{q} + \frac{1}{\delta_n} \qquad (1)$$

where δ_n, the cross-linking coefficient, is the average number of cross-linked units per number average molecule, and p and q are the fracture and cross-linking densities respectively. Assuming that p and q are proportional to the radiation dose r, then the sol fraction is related to the densities of fracture per unit dose (p_0) and cross-links per unit dose (q_0), by the equation:

$$s + s^{1/2} = \frac{p_0}{q_0} + \frac{1}{q_0\, u_1\, r} \qquad (2)$$

where u_1 is the number average degree of polymerization. Charlesby and Pinner applied their treatment to experimental results on polyethylene, polyvinyl acetate and other polymers.

Expression 1 may be applied without making assumptions regarding the dependence of p and q upon the radiation dose.

Lightly masticated pale crepe rubber, which had been freshly extracted with acetone, was evacuated and sealed in glass tubes. After irradiation, with gamma radiation from a cobalt-60 source or a linear electron accelerator, the soluble fraction was measured by extraction of the samples with benzene. In each case, the mean intensity of the radiation was estimated by ferrous sulphate dosimetry ($G_{Fe(3+)} = 15{\cdot}6$).

Solubility data for the gamma irradiation of rubber at two dose-rates at 25°C., quantitatively analysed in the manner adopted by Charlesby and Pinner, are shown in Fig. 1. Experimentally, the $s + s^{1/2}$ values diverge from the straight line drawn through data relating to high doses. Presumably the initial distribution of molecular weights is not perfectly random and some dislinking is necessary to attain this.

Fig. 1 shows that the rate of cross-linking is directly proportional to the intensity of radiation, similar to the behaviour of polyethylene[2] and other polymers. The G-value of the cross-linking reaction, calculated

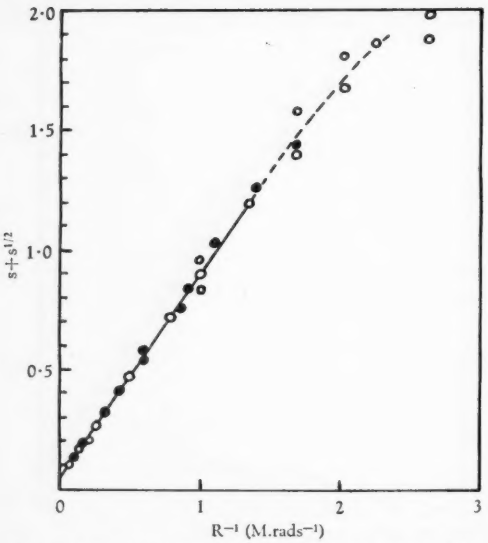

Fig. 1. Curve of $s+s^{1/2}$ against reciprocal dose for rubber at 25°C. ○, Dose-rate, $5{\cdot}5\times10^{4}$ rads/hr. ; ●, dose rate $=5{\cdot}1\times10^{4}$ rads/hr.

from the slope of the straight line in Fig. 1 and the osmotic molecular weight of the rubber (683,000), is 0·83 at 25°C.

The ratio of the rate of dislinking to cross-linking (p/q), determined from the intercept, is 0·05 at 25°C., in fair agreement with estimations by alternative methods[3].

Infra-red spectroscopic analysis revealed a marked increase in the absorption near 1,639 cm.$^{-1}$, identified as terminal unsaturation, presumably arising from the dislinking reaction. There was also evidence of the formation of *trans*-vinylene unsaturation during irradiation, manifested by the appearance of an absorption band at 980 cm.$^{-1}$, similar to that observed on sulphur-vulcanization of rubber[4].

I wish to thank Miss M. A. Place, of the British Rubber Producers' Research Association, Welwyn Garden City, for osmotic molecular weight measurements.

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Application of the Mass Spectrometer to Polysaccharide Chemistry

During work on the dissociation of certain classes of organic molecules induced by electron-impact[1], the field has been extended to some members of the carbohydrate series[2]. Using a newer technique[3] we have obtained a spectrum of a polysaccharide derivative. A methylated polysaccharide—methylated laminarin—was chosen for this study, since previous experience[4] has shown that methylated derivatives of carbohydrates are most suitable for this type of work.

Table 1. Mass-spectrum of Methylated Laminarin

m/e	27	28	29	31	32	39	40	41	42	43	44
rating	w	vs	m	m	s	w	vw	m	w	s	m
	45	55	56	57	60	67	68	69	70	71	82
	vw	m	w	m	w	w	vw	m	vw	w	w
	83	84	85	86	95	97	99	107	108	110	111
	vw	w	vw	w	vw	vw	vw	w	w	m	vw
	112	124	129	131	143	144	145				
	m	w	m	w	w	vw	w				
	156		188		230	268	296				
	m		m		m	w	w				
	364	367	395	397	434	490	493	500	515	532	550
	m	w	m	m	s	w	w	w	w	w	w
	566		595		635	644	656				
	w		s		s	s	s				

vw. very weak, w. weak, m. medium, s. strong, vs. very strong.

As can be seen from Table 1, the spectrum obtained can be considered to consist of two main parts: (*a*) a series of peaks of medium to strong intensity corresponding to fragment ions having m/e ranging from 28 to 156 ; and (*b*) a series of rather widely spaced peaks of weak to medium intensity which extend from m/e 152 up to 700. In this experiment scanning above 700 was not attempted. From work[4] already carried out on the 'cracking patterns' of methylated monosaccharide derivatives, part (*a*) of the spectrum is recognizably associated with the decomposition induced by electron impact of individual methylated sugar units ; it will not be discussed further here.

Part (*b*) of the spectrum gives a picture of the fragmentation of the polymer. There appears to be a number of series of fragment ions being produced, but on examination of the m/e values, patterns can be distinguished for two main series (188, 395 and 595 ; 230, 434 and 635). These two series have an approximate mean difference of 204, and a third though weaker set of peaks in the spectrum (156, 364 and 566) can also be considered as having the same separation. It appears significant that this mass factor of 204 is the same as the mass of a 2:4:6-tri-O-methyl glucose residue of which methylated laminarin[5] is mainly (at least 85 per cent) composed. These observations are consistent with the view that stepwise fragmentation of the polymer is taking place.

The ions (m/e 156, 188 and 230) corresponding to the first members of the three fragmentation patterns mentioned probably arise from the end-groups of the methylated laminarin. For example, it is not unreasonable to suggest that the ion of m/e 230 comes from the tetra-O-methyl glucose residue (molecular weight 235) at the non-reducing end of the polymer. This aspect of the problem, together with further extensions of this work, will be discussed more fully at a later date.

We thank Prof. E. L. Hirst and Prof. R. A. Raphael for their interest and encouragement ; and Mr. J. M. Wilson for technical assistance. One of us (P.A.F.) thankfully acknowledges the award of an I.C.I. Fellowship by the University of Edinburgh.

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RADIOCHEMISTRY

Physical and Chromatographical Observations of γ-Irradiated Potato Starch Granules

THE changes produced in potato starch by the action of ionizing radiation is of considerable importance and have been the subject of many investigations in recent years[1-4]. Physical changes induced in air-dried starch granules by γ-irradiation indicated that hydrolytic degradation had been occurring. Therefore, small molecular fission products of an irradiated specimen of potato starch were analysed by several chromatographical methods.

Air-dried potato starch granules containing 18·5 per cent moisture were irradiated from a source of 1,400 c. cobalt-60 giving total doses of 10^5–10^6r. at a rate of $2{\cdot}5\times10^5$r./hr.

As a result, intrinsic viscosity, viscoelasticity measured by a V.I. viscometer, and blue value of the starch specimens decreased, while reducing power, alkali lability number, carboxyl value, carbonyl number and susceptibility by β-amylase of the specimens increased corresponding to radiation doses. X-ray diffraction diagram showed that the crystalline part of the potato starch is damaged by doses up to $1{\cdot}5\times10^7$r.

From an 80 per cent ethanol extract of the irradiated starch specimen, D-glucose, maltose a pentose D-glucuronic acid, D-gluconic acid, and a series of small dextrins were detected by paper chromatographic analysis.

A quantitative separation of sugars from an alcoholic extract of 6×10^7r. γ-irradiated potato starch grunules, was carried out by the elution of their borate complexes from strong-base anion

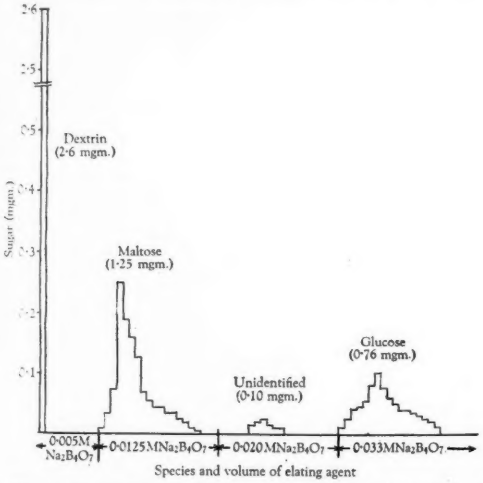

Fig. 1. Separation of glucose and maltose from γ-irradiated starch granules (1·8 gm.). Total dose, 6×10^7r., exchanger 0·6 cm.²×6 cm. Strong-base anion resin ('Dorvox-1-8X'). 300 mesh, borate form. Eluting agent borate solutions. Flow-rate, 1 ml./min.

exchangers with boric-borate solution[5-7]. D-glucose and maltose were separated readily from other sugars and measured by the anthron method[8]. The results were shown in Fig. 1. Gases formed during γ-irradiation were analysed using gas chromatography by nitrogen elution from an absorption column packed with silica gel, and hydrogen, carbon monoxide and carbon dioxide were identified.

Thus it is assumed that ether bonds of the starch molecule are split into corresponding radicals, then these are combined with H and OH radicals of crystallization or the starch molecule itself forming mono- and oligo-saccharides. Sugar acids and a pentose might be formed secondary from D-glucose.

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BIOCHEMISTRY

Pantothenic Acid Deficiency and Ubiquinone Levels in Rat Liver Mitochondria

A NEW group of naturally occurring homologous quinones referred to as ubiquinone or coenzyme *Q* (Q_{275}) has been recognized in recent years[1]. Structural studies have led to the characterization of these compounds as derivatives of 2, 3,-dimethoxy, 5-methyl benzoquinone, substituted at position 6 with a polyisoprenoid side-chain[1,2]. Homologues with 6–10 side-chain isoprenoid units have been identified in animal and plant tissues and in micro-organisms[3]. While much interest has centred around its biological functions, little work has been done on the biosynthesis of ubiquinone. The incorporation of mevalonic acid into the isoprenoid chain has been recently reported[4]. The known participation of coenzyme *A* and acetate in the biosynthesis of mevalonic acid prompted a study of the possible relationship of dietary pantothenic acid to tissue-levels of ubiquinone.

Weanling rats (40–50 gm.), Wistar strain, were divided into two groups of six each, one receiving the basal diet consisting of an 18 per cent purified casein ration deficient in pantothenic acid and replete in all other vitamins and the other receiving the same diet supplemented with calcium pantothenate at a level of 20 mgm./kgm. diet[5]. The animals were killed at end of 10 weeks, by which period those on the basal diet showed typical symptoms of pantothenic acid deficiency. Livers were excised, chilled in cracked ice and a 20 per cent homogenate prepared in ice-cold isotonic (0·25 *M*) sucrose; an aliquot was used for centrifugal fractionation of mitochondria[6].

The unsaponifiable lipids from mitochondria were extracted with ether, chromatographed on Brockmann grade alumina and ubiquinone determined in the appropriate fraction spectrophotometrically by measurement of the characteristic absorption at 272 mμ in the oxidized and reduced states[4]. Coenzyme *A* was assayed by acetylating ability using pigeon liver enzyme (cf. ref. 5). Pantothenic acid was determined microbiologically using *Lactobacillus arabinosus*[8]. Vitamin B_{12} was assayed with *Euglena gracilis*[9] after liberation by overnight incubation with papain under toluene. Succinoxidase activity was followed manometrically in the Warburg apparatus.

It will be seen from Table 1 that rats on the basal diet have decreased hepatic stores of total pantothenic acid and of coenzyme *A* (cf. ref. 5). Pantothenic acid deficiency results in a marked fall in the concentration

Table 1

Group	Gain in weight (gm./week/rat)	Pantothenic acid (μgm./gm.)	Coenzyme A (units/gm.)	Ubiquinone ((μgm./gm.)	Succinoxidase activity (μl. O_2/hr./gm.)	Vit. B_{12} (mμgm./gm.)
Pantothenic acid-deficient	10·2 ± 0·8*	86·4 ± 3·2	113 ± 6	36·0 ± 2·3	1175 ± 51	155 ± 8·2
Pantothenic acid-fed	14·6 ± 0·4	127·0 ± 5·9	157 ± 9	44·5 ± 2·1	1343 ± 39	78 ± 6·6

* Standard error of the mean. Results are on wet weight basis.

of mitochondrial ubiquinone as also in mitochondrial succinoxidase activity. The decrease in ubiquinone parallels the fall in succinoxidase activity and is consistent with the reported participation of ubiquinone in electron transfer systems in general and in the succinoxidase system in particular[10].

The decreased liver stores of vitamin B_{12} in the animals fed pantothenic acid points to the reciprocal relationships recognized between these two vitamins (cf. ref. 11). Other studies have also shown that the rapid depletion of liver stores of vitamin B_{12} in experimental thyrotoxicosis[12] is attended by elevated levels of ubiquinone and succinoxidase activity in liver mitochondria (unpublished data).

Further studies are in progress.

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Histidine-Copper (II) Complexes

Stability constants are known for copper complexes of most of the naturally occurring α-amino-acids. However, although it is of considerable biochemical interest, the copper–histidine system has not been interpreted in detail. The system is anomalous, probably because, although the metal can co-ordinate to the amino, imidazole or carboxyl groups in histidine, an assumed planar configuration of the cupric complexes allows only two sites in any histidine molecule to be bound simultaneously[1]. In this way both the histidine anion (L^-) and the zwitterion (HL^{+-}) can act as chelating species, giving the five different complexes, CuL^+, $CuHL^{++}$, CuL_2, $CuHL_2^{++}$ and $Cu(HL)_2^{++}$. Because the concentration ratio of L^- to HL^{+-} is dependent on pH, the proportions of the complexes present will vary with both ligand concentration and pH.

Recently, stability constants have been reported[2] for CuL^+ and $CuHL^{++}$ and for a complex suggested to be Cu(OH)L. Evidence for this complex was the second linear portion observed in the titration curve of 1 : 1 copper–histidine mixtures (Fig. 1), which was consistent with the formation of such a complex with $pK = 7{\cdot}19$. However, titration curves of this type, and apparent pK's of about this value which are obtained from them, are very common among 1 : 1 complexes with bidentate ligands and, as will be discussed fully elsewhere, it can be shown by mathematical analysis that in many cases the reaction taking place is actually a dismutation into the normal 1 : 2 complex and cupric ion, which then hydrolyses to $CuOH^+$ and $Cu(OH)_2$. This seems to be the case with the copper–histidine system because precipitation occurs if a weakly alkaline solution is allowed to stand.

If this interpretation is correct and the assumption is made that the only 1 : 2 copper–histidine complex present in neutral or alkaline solutions is CuL_2, results for the 1 : 1 system lead to an estimate of log $\beta_2 \sim 19$ where $\beta_2 = [CuL_2]/[Cu^{++}][L^-]^2$. This estimate provides a starting point for an iterative process by which all three stability constants of the 1 : 2 complexes can be calculated. From the titration curve in Fig. 1 for a 2 : 1 copper–histidine mixture, the stability constants log $K_1 = 10{\cdot}35$ and log $K_1 = 5{\cdot}07$ were obtained for CuL^+ and $CuHL^{++}$, in good agreement with Leberman and Rabin's values[2]. (As our concentrations were very different, polynuclear complex formation does not appear to have been significant.) Using these results, and acid dissociation constants (pK's) for histidine of 1·82[1], 6·02 and 9·20,

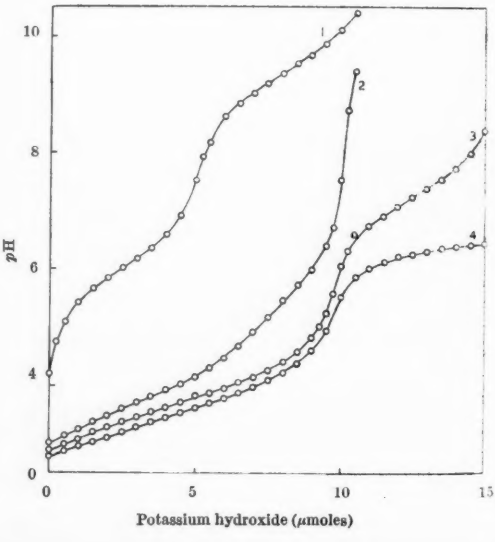

Fig. 1. Titration curves of histidine hydrochloride (5·0 m*M*) containing indicated cupric nitrate concentrations, with potassium hydroxide (0·1 *M*): 1, none; 2, 2·5 m*M*; 3, 5·0 m*M*; 4, 10·0 m*M*

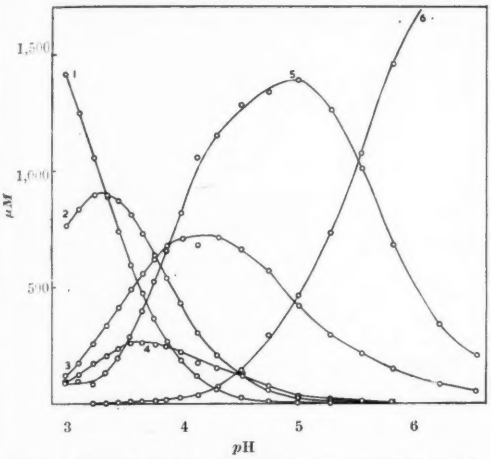

Fig. 2. Calculated variation with pH of the composition of the 1 : 2 copper, histidine system (curve 2) in Fig. 1 : 1, Cu++; 2, CuHL+; 3, CuL+; 4, $Cu(HL)_2$++; 5, $CuHL_2$+; 6, CuL_2

it was possible to analyse quantitatively the titration data for 1 : 2 copper–histidine mixtures. The values obtained were $\log \beta_2 = 18{\cdot}70$, $\log \beta_2' = 24{\cdot}33 - p\text{H}$, and $\log \beta_2'' = 27{\cdot}90 - 2\ p\text{H}$, where $\beta_2' = [CuHL_2]/[Cu^{++}][L^-]^2$, and $\beta_2'' = [Cu(HL)_2]/[Cu^{++}][L^-]^2$. With these values of β_2 and β_2'', $\log \beta_2' + p\text{H}$ was constant within $\pm 0{\cdot}1$ over the range pH 3·38 to pH 6·71. These results confirm that in neutral and alkaline solutions only the complexes CuL^+ and CuL_2 are present in significant amounts, and that the second linear portion in the 1 : 1 system is due to a dismutation reaction.

Fig. 2 shows how the composition of a 1 : 2 copper histidine mixture varies with the pH of the solution.

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A Two-Dimensional System for the Separation of Amino-Acids and Peptides on Paper

A rapid, reproducible, semi-quantitative assay of the amino-acid composition of peptides is of great value in amino-acid sequence studies. Many of the common techniques of paper chromatography present difficulties in the subsequent quantitative estimation of the amino-acids. Dixon *et al.*[1] have described the separation of amino-acids by use of high voltage paper electrophoresis at pH 3·6, followed by paper chromatography in *n*-propanol-pyrophosphate buffer. Using electrophoresis at pH 2·2 (4 per cent v/v formic acid, 0·3 per cent pyridine), followed by chromatography in *n*-butanol; *n*-butyl acetate; acetic acid; water (19:1:5:25), we have obtained a separation of the amino-acids (Fig. 1) which is rapid and reproducible. We have also found this system useful in the comparison of peptides from a series of proteolytic digests.

Electrophoresis at pH 1·9–2·3 gives good separation of serine, glycine and aspartic acid; of methionine, valine and tryptophan; and of glutamic acid, threonine and alanine. It therefore complements the well-known separation by butanol-acetic acid chromatography, which resolves aspartic acid, methionine sulphone, tryptophan and phenylalanine. Proline and tyrosine are resolved by the butanol-butyl acetate mixture in the same way as with the 'aged' butanol-acetic acid solvent described by Levy and Chung[2]. Leucine and isoleucine are resolved in this system only after development for 24 hr.

We have adopted the following experimental procedure: Acid-free hydrolysates of 0·02–0·20 μmoles of peptide are applied as 1 cm. bands, 2 cm. apart, 15 cm. from the bottom (anode) of 58 × 20 cm. Whatman No. 3 or 3 *MM* paper. An amino-acid marker is run in parallel on the same sheet. After wetting in the usual way with pH 2·2 buffer, the sheets are subjected to electrophoresis under toluene at 2,000 V. for 70 min.[3] When dry, the amino-acids are located by spraying the marker strip with ninhydrin. Cysteic acid runs approximately 8 cm. toward the anode. Lysine is well separated, running 36 cm. toward the cathode, while histidine and arginine overlap at about 34 cm. from the origin. The basic amino-acids can be further resolved, if necessary, by stitching appropriate areas to strips of the same paper and running high voltage electrophoresis at pH 6·5[4] (Fig. 1).

The remaining amino-acids are contained in a strip approximately 12–30 cm. from the origin. Such strips are cut out and stitched, with a sewing machine, along either side to a 6 cm. and a 50 cm. paper of the same width. These papers are then run for 16–24 hr. in the descending butanol-butyl acetate solvent. Fig. 1 shows a typical chromatogram.

The amino-acids may be detected with a dilute ninhydrin spray and estimated by the method of Hanes and co-workers[5]. In this way the amino-acid

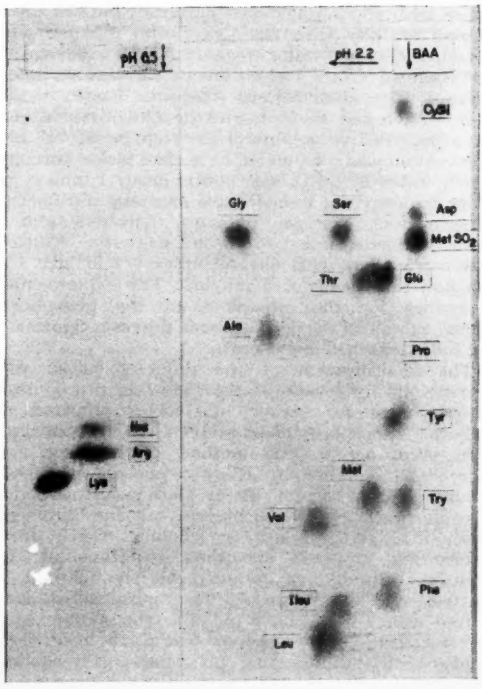

Fig. 1

content of 0·1 μmole of peptide may be rapidly determined to an accuracy of about 10 per cent.

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Phospholipid Phosphoric Esters in the Human Placenta

According to earlier studies of Watanabe[1] the phospholipids, chiefly lecithin, account for nearly 30 per cent of the total lipids in the human placenta, but the metabolism of these compounds in this tissue is not well known. So far only the synthesis of fatty acids has been supposed to occur in the epithelial cells of the placenta.

In the present study an ion-exchange chromatography technique[2] has been introduced for the separation, identification and determination of the phospholipid phosphoric esters in human placenta.

The placenta of healthy women at the end of gestation, freed from blood and connective tissue, was excised rapidly, dropped into ice-cold distilled water (about 100 ml.), homogenized and boiled at *p*H 4·8–4·9 (0·5 *N* hydrochloric acid) for a few minutes. The protein-free almost colourless filtrate was dialysed, the diffusate then concentrated *in vacuo* to a volume of about 2–3 ml., brought to *p*H 2·0–2·5 with 0·2 *N* hydrochloric acid and chromatographed on a column of 'Zeo-Karb 225' Na^+ form, 4·5 per cent cross-linked (1·8 water regain), under 200 mesh. The resin was in the form of microbeads with a mean value of 25 μ of diameter. The total length of the resin column was 168·5 cm. and the diameter 1·25 cm., the total effective volume of the resin being 213 ml. The column was surrounded by a glass jacket through which water at 23°C. was continuously running, in order to keep the temperature constant during the experiments. Nitrogen pressure, delivered from a cylinder, corresponded to 20 cm. mercury. Elution was carried out with citrate buffer 0·2 *M* *p*H 3·1, the flow-rate being of 5 ml./hr. The experimental procedure for the separation of the phosphoric esters, as well as the details about the resin treatment are fully described elsewhere[2].

The identification of the different peaks was carried out by means of the following procedures: (*a*) reference to marker substances, prepared as follows: phosphorylcholine (PC) as the calcium salt, according to the method of Plimmer and Burch[3], as modified by Riley[4]; phosphorylethanolamine (PE) as barium salt by Outhouse's method[5]; phosphorylserine (PS) as barium salt by Plimmer's method[6]; glycerylphosphorylcholine (GPC) from ovolecithin prepared according to Hanahan[7] on alumina columns, or obtained by the method of Tattrie and McArthur[8]. The pure substances, before using, were converted into the sodium salts by treatment with a proper 'Zeo-Karb' resin; (*b*) analysis of the inorganic phosphates (Pi)/choline, and Pi/NH_2-N ratios of the solutions corresponding to the various peaks, compared with those of the pure compounds; (*c*) paper chromatography of the desalted and concentrated peak solutions; small columns of 'Dowex 50' × 4 in H^+ form, under 200 mesh, were used for desalting; (*d*) analysis of the hydrolysis products of each peak and their identification by means of paper chromatography; (*e*) enzymic assay of the peak materials by means of a phosphomonoesterase, purified to the 5th step from intestinal mucosa, according to Morton[9] and by the use of a glyceryl-phosphorylcholine-phosphodiesterase, prepared from rat liver according to Dawson[10]. Our preparation of phosphomonoesterase had a specific activity of 12·300 units/mgm. N and the K_m for synthetic PE and respectively PC were $4{\cdot}075 \times 10^{-3}$ and $1{\cdot}185 \times 10^{-2}$ at 38°C. The GPC-phosphodiesterase, practically free from monophosphoesterase activity, showed for synthetic GPC a K_m of 1×10^{-3} *M* at 37°C.

Determinations of NH_2-N, choline and Pi of the separated fractions were carried out essentially according to the methods of Jacobs[11], Webster[12] and Strickland *et al.*[13], respectively. Choline was determined from the peak containing GPC after 1 hr. of hydrolysis at 100°C. and from the peak containing PC after 48 hr. at 123°C. This technique (Baker and Porcellati[2]) enables the absolute amounts of the two substances (PC and GPC) to be calculated in the presence of NH_2-N-containing phosphoric esters, which were eluted from the resin column closely to some others.

By means of these procedures the following phospholipid phosphoric esters have been identified in human placenta: GPE, GPC, PE and PC. The results are given in Fig. 1, which represents one example of several experiments. The peaks are almost all resolved on the column with only some overlapping between GPC and GPE, and PC and PE. No evidence has as yet been obtained on the presence of PS in the extract, because a correct identification of this compound has not been possible. Some other NH_2-N-containing compounds have been found, as taurine, urea, aspartic acid, threonine, serine, glutamic acid, together with some other small peaks, one of which (fractions 138–142) has been identified as belonging to a phosphopeptide of unknown composition, similar to that previously found in nervous tissue[2].

In Table 1 are reported the quantitative values in μmol./100 gm. wet weight of tissue of the phospho-

Table 1. Concentration of Phospholipid Phosphoric Esters in Human Placenta and in Avian Brain. Values in μmol. per 100 gm. Fresh Tissue, except for the P'peptide, for which they are referred as μmol. Leucine Equivalent

Substance isolated	Placenta	Brain
Phosphorylserine	—	7·5
Glycerylphosphorylethanolamine	26·9	83·4
Glycerylphosphorylcholine	18·4	18·9
Phosphorylethanolamine	49·6	139·0
Phosphorylcholine	7·8	70·1
Phosphopeptide	6·8	not calculated

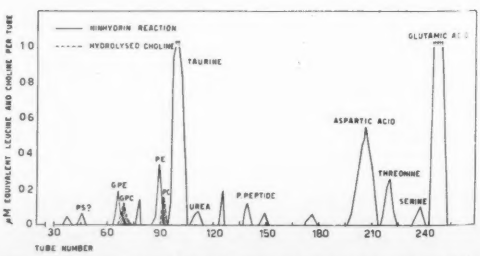

Fig. 1. Ion-exchange chromatogram of human placenta extract from 4·8 gm. tissue

peptide, together with those of PE, PC, GPE, GPC and PS, compared with the results of the avian brains[2].

The results show that the values of the phospholipid phosphoric esters (except GPC) are lower in placenta than in brain tissue. On the contrary the NH_2-N-containing compounds (not reported in Table 1) are lower (except aspartic acid) in brain than in placenta extracts.

The presence of the phospholipid phosphoric esters in human placenta lead us to suppose that the metabolism of the phospholipid molecules is operative in this tissue.

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Extraction and Purification of 'Queen Substance' from Queen Bees

Queen honeybees (*Apis mellifera*) secrete material which, distributed through the colony, affects the bees in two ways, inhibiting the development of ovaries in workers and influencing their behaviour by inhibiting queen rearing (that is, queen-cell construction)[1]. Carlisle and Butler[2] observed similarities between the 'queen substance' of honeybees in the form of an alcoholic extract and the ovary-inhibiting hormone of prawns (*Leander serratus*), and it is a justifiable extrapolation to suppose that this material has important physiological effects on animals of other phyla.

A method of quantitative assay based on the inhibition of the construction of emergency queen cells was devised by Butler and Gibbons[3], and used by Butler and Simpson[4], to demonstrate that 'queen substance' is contained in the mandibular glands of the queen honeybee. Using this test, the extraction and concentration of 'queen substance' have been investigated and a crystalline material of high activity has now been isolated.

The head of the queen bee is a convenient raw material for extraction, and, after solvent extractions and partitions, an acidic fraction is obtained which is finally purified by chromatography. Quantities are given for 100 heads of mated queen bees. These were extracted in a Soxhlet apparatus with ethanol for 4 hr., the residue from the extract, about 150 mgm., was taken up in ether and water, the solutions were separated and the aqueous layer extracted with ether until a clear ether extract was obtained; the dried ethereal solution yielded approximately 90 mgm. The extract was partitioned between 6 ml. of 50 per cent aqueous ethanol and 6 ml. of light petroleum (boiling point 80–100°) and the petroleum layer was extracted again with 6 ml. of aqueous ethanol. The residue from the aqueous ethanolic solution, approximately 60 mgm., was taken up in 6 ml. of ether and extracted three times with 6 ml. of *N* sodium hydroxide. The alkaline extract was saturated with carbon dioxide, extracted with ether, acidified and again extracted with ether. The more strongly acidic fraction was separated into two fractions by chromatography on paper (stationary phase, water/methanol (1:3), mobile phase toluene). The fraction with R_F approximately 0·5 was cut out and eluted with ethanol. The ether-soluble fraction of the extract was extracted into phosphate buffer *p*H 8·3 which was acidified and extracted with ether.

An extract obtained in this way (18 mgm.) yielded waxy crystals, melting point 45–50°, which could be recrystallized from aqueous methanol. The characteristic infra-red absorption spectrum (Fig. 1), paper-chromatographic behaviour, potentiometric titration and colour reactions indicate that the crystalline material is an αβ-unsaturated carboxylic acid, containing an unconjugated carbonyl group and with a molecular weight of the order of 190. It would appear to be closely related to the 10-hydroxy-Δ^2-decenoic acid present in royal jelly[5] and secreted in the mandibular glands of worker bees[6]. Tested on groups of bees, about 0·13 μgm. per bee inhibited queen-cell production. The investigation is being continued and results will be published in detail elsewhere.

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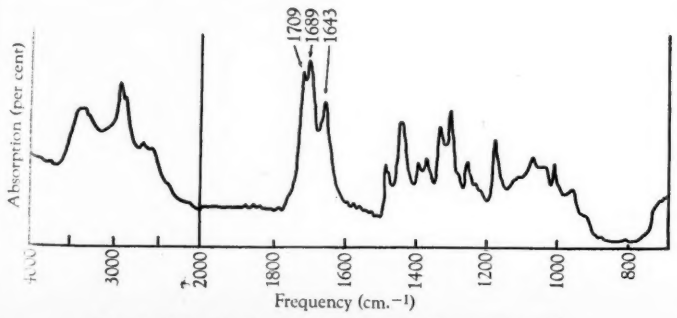

Fig. 1. Infra-red absorption spectrum of 'queen substance'. 160 μgm. in 40 mgm. potassium chloride. Perkin-Elmer Model 21; sodium chloride prism

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PHYSIOLOGY

Sterol-Balance Experiments in Humans

The application of phytosterols and especially of sitosterol for the therapy of hypercholesterolæmia[1] has brought up again the question of the absorption of sterols. Surprisingly, not many sterol absorption experiments in humans have been performed until now with the exception of absorption experiments with cholesterol[2–4]. We therefore examined the apparent absorption in humans of β-sitosterol, mixed soya sterols, stigmasterol acetate, sitosterol stearate, and cholesterol.

Cholesterol (Merck, Darmstadt, m.p. 149°C.), mixed soya sterols (Mann, Research Laboratories, New York, m.p. 133°–141°C.), stigmasterolacetate (Mann Research Laboratories, New York, m.p. 138°–146°C.), sitosterol (Bœhringer and Söhne, Mannheim, m.p. 132°–134°C.), and sitosterol stearate (m.p. 72–77°C.) were given to subjects in the form of a 20 per cent emulsion in 3 per cent carboxymethylcellulose. The diet of the subjects was accurately standardized during the time the experiment taking place. 5–10 grams of the emulsified sterol were given daily and the fæces were collected before, during and after the sterols were given to the subjects.

The wet fæces were weighed and then placed in acetone. Shortly thereafter the fæces were extracted quantitatively with acetone in a Soxhlet extractor. An aliquot part of the total lipid extract was then saponified and extracted by means of petroleum ether. Ether was used for extracting the saponifiable fraction after acidification. Both fractions, unsaponifiable and saponifiable, were estimated gravimetrically. In the unsaponifiable fraction 3β-hydroxysterols were precipitated by means of digitonine.

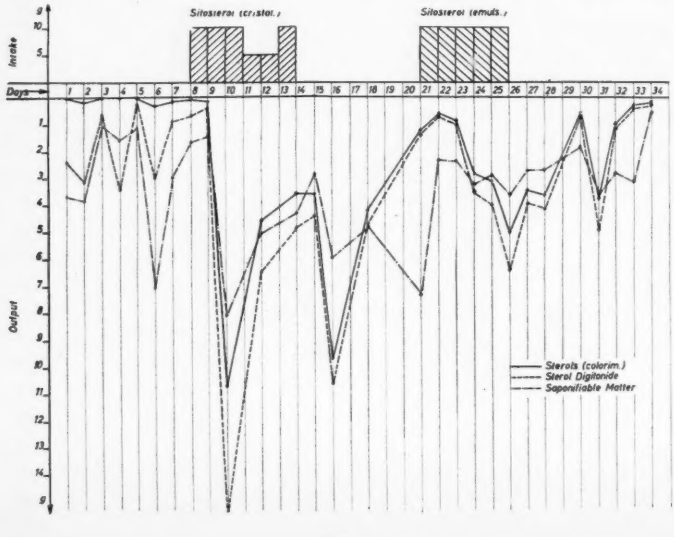

Fig. 2

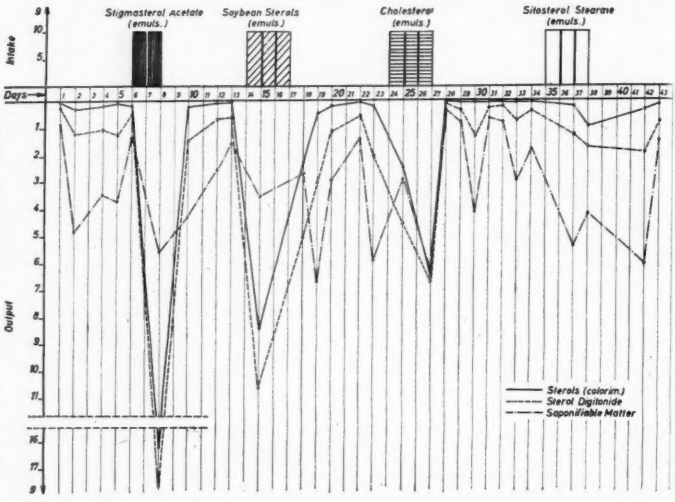

Fig. 2

The unsaturated sterols were also determined colorimetrically by the Tschugaeff reaction. In addition, the sterol esters were isolated from the total lipid extract by a chromatographic method and the colour-producing sterols were estimated.

Bacterial disintegration of sitosterol was controlled by anaerobic incubation of suspensions of fæces. In these experiments we could recover the previously weighed material. For measuring the sterol output, we used gravimetric determinations.

After feeding 30 gm. of sitosterol (Fig. 1) in crystalline form, we recovered in the fæces practically all the sitosterol with the sole exception of a small part of the sterol. By feeding the subject the 20 per cent emulsion of sitosterol, there was a difference of 55 per cent between the intake and the output. The amount of sterol esters in the fæces averaged 18 mgm./100 gm. during the time immediately preceding the feeding period and this amount grew tenfold during the feeding period. The difference of apparent absorption of the two sitosterol preparations is not due to bacterial disintegration of the sterol caused by the finer dispersion of the emulsified sitosterol. It is believed that after 48 hr. of anaerobic incubation of a fæces suspension with emulsified sitosterol there was no loss of the sterol content.

The experiment on feeding stigmasterol acetate, soya sterols and sitosterol stearate was the first time this has been carried out on a human being (Fig. 2). Cholesterol was used for comparison. The sterols were given as 20 per cent emulsions. The apparent absorption of soya sterols was found to be 48 per cent and the absorption of sitosterol (Fig. 1) and cholesterol were found to be 55 per cent each. The varying results which are produced after feeding stigmasterol acetate are due to a faster passage through the

intestinal tract caused by diarrhœa. For this reason we were able to re-extract 11·1 gm. of sterol esters from the fæces. The apparent absorption in this case was 20 per cent. After adding 30 gm. of sitosterol stearate (equal to 18 gm. sitosterol) to the subjects' diet, we were able to recover only 3 gm. of sterols. In all these experiments we observed an increased ester fraction equalling approximately five times that of the control.

As regards cholesterol absorption, our values are in agreement with former estimates[2,3,5]. In the case of sitosterol the experiment performed by R. G. Gould[6] shows an absorption of 5 per cent; R. P. Cook was unable to recover 40 per cent of sitosterol in feeding experiments on humans. Our results indicate that the amounts of the sterols absorbed depend on the size of the sterol particles. Contrary to the results of experiments on animals by D. W. Peterson[8] and by L. Swell[9], we found an increased apparent absorption after feeding sitosterol stearate. Due to reasons beyond our control, we were unable to carry out the experiment with stigmasterol acetate.

Our results indicate that emulsified sterols given to subjects are absorbed in the same quantity as cholesterol, but like cholesterol not quantitatively.

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Fusion Frequency and Light Quantity

WHEN an intermittent photic stimulus alternates at an increasing rate it is found, in general, that the visual apparatus resolves the alternations below, but fails to do so above, a critical rate. This rate is called the fusion or critical flicker frequency.

Within limits, the fusion frequency varies linearly with the logarithm of the light intensity (Ferry–Porter Law[1]) and with that of the areal subtense (Granit–Harper relation[2]). It has recently been shown that, for the rod-free area of the retina, the operative variable is the light quantity[3]. Collins's results[4] lead to a similar conclusion. Centrally fixated fields[5] obey this rule rigorously up to a diameter of 7·5° if the receptor number rather than stimulus area is considered[6]. Best[7], working with 1° and 10° fields observed a breakdown of the rule, a finding consistent with that deduced from the data of Kugelmass and Landis. Do rods obey the rule when cones are not stimulated.

To answer this point, earlier experiments[3] were repeated and extended to a region lying 6° below the retinal fixation area. Light obtained from a d.c. car-lamp was filtered with Ilford 624 and 625 (green) filters in series so as to obtain a band providing stimuli approximately equal for rods and cones. A rotating sector disk (90°) interrupted the stimulus regularly, at a rate which was under manual rheostatic control and read off a tachometer coupled to its axle.

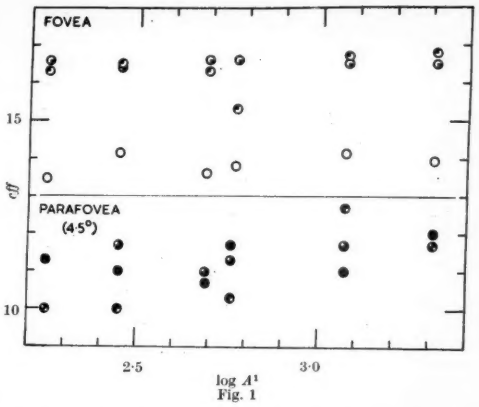

Fig. 1

The relation between threshold and stimulus area was determined for each observer for the foveal and para-foveal regions after periods of 5 and 25 min. dark adaptation respectively. In these experiments the exposure time was 0·04 sec., in the flicker experiments 1 sec. The angular diameter of six test fields ranged from 18′ to 57′ The flicker measurements were made at various intensities increased above the threshold by a factor M, lying between 0·2 and 1 log units. Fig. 1 shows results obtained for three observers at a stimulus-level corresponding to 0·3 threshold units. The different symbols refer to different observers, the white ones representing the fovea, the black ones the periphery. The mean fusion frequency corresponding to this level is approximately 15·5 c./sec. for the fovea and 11·2 c./sec. for the para-fovea. Within the limits of the experiment, therefore, the effective quantity of light is found to govern the fusion frequency of both rods and cones.

Two observers determined the fusion frequency as a function of M, multiples of the threshold quantity of light. Their foveal data agree, but their para-foveal data tend to diverge for larger values of M. Extrapolation towards $M = 0$, suggests that, under the conditions of this experiment, the fusion frequency at the threshold is approximately 11 c./sec. for the foveal cones and 8 c./sec. for para-foveal rods.

The present confirmation and extension of the earlier data[3] supports the view that, although central factors may have a bearing on the fusion frequency[8], the portion of the visual path which fails to follow stimulus alternation at a given level must lie in the retina. Additional evidence is obtained from a consideration of the results of Østerberg[9], Kugelmass and Landis[5], Hecht and Verrijp[10]. When the independent variable in the results of Kugelmass and Landis is changed from stimulus area to number of cones ν covered (and presumably stimulated) by the flickering test field a new set of data is obtained. The slope $\partial n/\partial \log \nu$ (where n is the fusion frequency) is of course steeper than $\partial n/\partial \log A$. For the three highest luminance levels examined, the slopes are parallel, the mean value being shown in Fig. 2. Hecht and Verrijp measured n as a function of retinal eccentricity at 0°, 10, 15 and 20° respectively. Østerberg's results show that the cone density varies appreciably in these parts of the retina, and it is interesting to note that when the suitable substitution is made in Hecht and Verrijp's data their variation is described approximately by the line culled from Kugelmass and Landis' work. Weekers and Roussel's[11] iso-frequency plots also support this conclusion. It follows that, within the

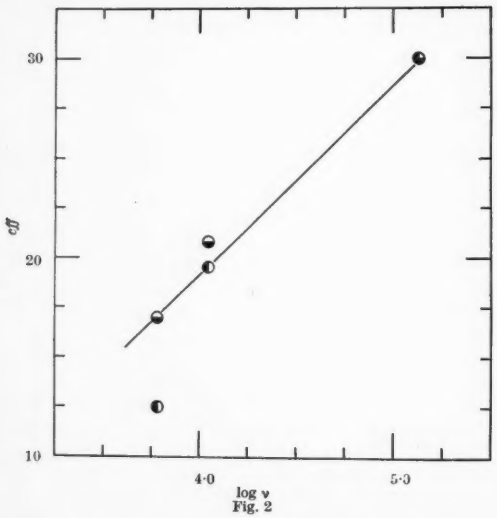

Fig. 2

limits of the experiments discussed, the relation between fusion frequency n, stimulus intensity I, and ν, the total number of cones stimulated, is given by:

$$n = k \log I\nu^{p} + k'$$

where k and k' are constants, and p is the index of retinal summation.

It should be mentioned that the difference between the fusion frequencies for rods and cones respectively, as illustrated in Fig. 1 and as extrapolated to the absolute threshold, cannot be accounted for in terms of the different number of receptors stimulated. Enroth[12] has shown that in a given cat optic nerve fibre there is some correlation between the fusion frequency on the one hand and the reciprocal of the latent period of (single flash) on-discharges and repetitive off-discharges respectively. It is noteworthy that the difference in the rod and cone fusion frequency near the absolute threshold, as found in the present experiments, correlates with a finding of Arden and Weale's[13], namely, that the rod latent period is longer than the cone latent period, also measured near their respective absolute thresholds.

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Specific and Sensitive Assay Method for Vasopressin and Oxytocin using Glass-Paper Chromatography

Although publications concerning measurement of vasopressin and oxytocin in blood and urine are legion, the lack of specificity of technique for concentration and bioassay of the trace amounts presumed to be present in biological fluids has led to wide variation in results and frank scepticism concerning their physiological significance[1]. Since bioassay techniques, although extremely sensitive, are inherently subject to wide error, a reliable quantitative method applicable to biological fluids demands a specific and sensitive technique for isolation and concentration of these polypeptides from blood or urine prior to bioassay.

Of the many methods reported for isolation of these hormones, that of Bissett *et al.*[2], utilizing acid-ethanol extraction of blood, shows fair recoveries, but suffers from lack of specificity since further purification is not performed. Heller *et al.*[3] recently reported a technique of filter paper chromatography for these neurohypophyseal polypeptides which could detect as little as 10 milli-units of activity by bioassay of eluted chromatograms.

Recovery of vasopressin was much better than oxytocin and this decay in activity may have been due to the fact that chromatography requires as long as 24–48 hr. for completion. In any case, the estimated concentration of these hormones in blood is in the order of micro-units rather than milli-units, which makes this method much too insensitive for physiological application. This communication reports a rapid and precise method for chromatographic separation of vasopressin and oxytocin which is sufficiently sensitive to be applicable to physiological and clinical investigations.

Chromatography is performed with glass-fibre paper strips 6 × 15 cm. long impregnated with silicic acid according to the method of Dieckert *et al.*[4] with the exception that the concentration of potassium silicate is is doubled here. Following impregnation, the papers are dried in an oven at 150–200° C. to increase their absorbant properties and stored in a desiccator prior to use to minimize water content, which greatly influences R_F. Two types of solvent systems were employed, one for vasopressin consisting of butanol, ethanol and ammonium hydroxide in a ratio of 60 : 30 : 10 (solvent A) and a second for oxytocin consisting of n-amyl alcohol and acetic acid, 80 : 5, saturated with water (solvent B).

Lyophilized commercial and purified or synthetic preparations of vasopressin and oxytocin are diluted in distilled water to requisite concentrations and 10 µl. aliquots spaced across the lower portion of a glass paper strip. The strips are placed in a covered glass jar of suitable size and lowered into the appropriate solvent for development by the ascending technique without preliminary equilibration. Chromatography is quite rapid, requiring about 30 and 50 min. respectively for solvents A and B. The chromatogram is dried with a fan at room temperature, sprayed from the rear with Folin's phenol reagent, and, after partial drying, then sprayed with saturated sodium carbonate. This reagent, which produces a blue colour in the presence of tyrosine, will detect as little as 0·5 µgm. polypeptide.

Using solvent A, synthetic oxytocin and 'Pitocin' run to the front while 'Pitressin' shows 5 spots at R_F 0·96, 0·71, 0·55, 0·40 and 0·00, with the spot at R_F 0·71 most clearly defined (Fig. 1). Only one spot

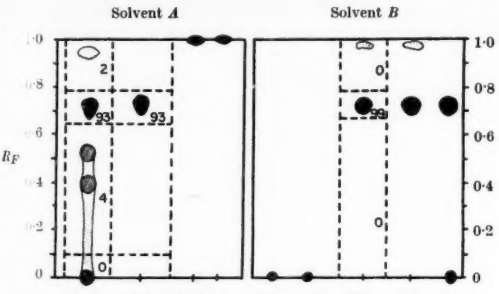

Fig. 1. Glass-fibre paper chromatograms of 'Pitressin' (*PT*), lysine vasopressin (*LVP*), synthetic oxytocin (*OXY*), 'Pitocin' (*PO*) and protopituitrin (*PP*). The shaded areas are proportional to the intensity of colour apparent with Folin's reagent. The numerals within the columns indicate per cent recovery

was observed in purified lysine vasopressin which ran to the same zone as the bulk of the material in 'Pitressin' (Fig. 1). When developed in solvent *B*, oxytocin shows 2 spots, one at the front which is very faint, and most of the material at R_F 0·71. 'Pitressin' does not move at all, and protopituitrin shows 2 spots at R_F 0·71 and 0·00 in this system.

For recovery experiments, 1·0 milli-units 'Pitressin' and synthetic oxytocin were developed on the paper in the appropriate solvents, the strips cut into various segments and each eluted overnight in the cold in 10 ml. of a solution containing sodium chloride, 8 gm., potassium chloride, 0·42 gm., Calcium chloride, 0·06 gm., sodium bicarbonate, 1·0 gm., sodium dihydrogen phosphate, 0·45 gm., and magnesium chloride, 0·1 gm. per litre. The theoretical concentration of 'Pitressin^R' and oxytocin in these solutions was 100 micro-units per ml. The activity of the eluates was tested by bioassay and compared with standard solutions.

Vasopressin was assayed by intravenous injection in alcohol-anæsthetized adult female rats using a modification of Dicker's method[5] and his electronic apparatus for constant hydration. In our hands, this method has been able to detect as little as 5 micro-units vasopressin/100 gm. Gaddum's superfused rat uterine strip method[6] was employed for the oxytocin assay using de Jalon's solution for the superfusion fluid. This method can measure as little as 3 micro-units oxytocin. Almost all the biological activity of chromatographed 'Pitressin' was recovered at R_F 0·71, the site of most of the chromogenic material and at the same R_F as lysine vasopressin (see Fig. 1). Synthetic oxytocin was also recovered quantitatively from the chromatogram at R_F 0·71, corresponding to identical zones observed in 'Pitocin' and protopituitrin.

Although one milli-unit quantities of vasopressin and oxytocin were arbitrarily selected for chromatography in the recovery experiments, the extreme sensitivity of these bioassay techniques suggests that, with minor modifications, this method should be applicable to measurement of physiological levels of these hormones in blood.

We are indebted to Mr. Robert Miller for technical assistance. 'Pitressin^R', 'Pitocin^R', and protopituitrin (12·7 pressor units and 20·2 oxytocic units/mgm.) were generous gifts from Dr. J. E. Gajewski, Parke, Davis and Co., synthetic oxytocin ('Syntocinon^R') was supplied through the kindness of Dr. J. D. Cuono, Sandoz, and purified lysine vasopressin (300 units/mgm.) kindly supplied by Dr. Roger Guillemin, Baylor University School of Medicine. This work was supported in part, by grants from the National Heart Institute of the United States Public Health Service (*H*–2850), the Louisiana and American Heart Associations and Upjohn Co.

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Effect of Protein Depletion on the Distribution of Protein Synthesis

Garrow, from observations on protein-deficient dogs[1] and human infants[2], advanced the hypothesis that in protein depletion the synthesis of protein is concentrated in the more essential organs at the expense of the less essential ones. He described this as a 'contradiction of the metabolic frontiers', which might be regarded as a compensatory mechanism. In Garrow's work measurements were made only on blood proteins, and there was no opportunity for the serial analysis of other tissues. Preliminary experiments on rats, in which measurements have been made on other organs, provide some support for Garrow's hypothesis.

Weanling rats were put on a simulated 'Jamaican' diet[3] containing 6 per cent protein. After 4–5 weeks on the diet they were injected intraperitoneally with approximately 15 μc. of methionine labelled with sulphur-35, and killed at intervals of 1, 2, 3, 5 and 7 days. Control animals on a stock diet (18 per cent protein) were treated in the same way. Blood was taken from the heart; the other organs were dissected out and dissolved in 1 *N* sodium hydroxide. The radioactivity and nitrogen content of these solutions were measured. In most cases, of the internal organs only liver and brain were analysed separately; the other organs (gut, pancreas, spleen, kidneys, lungs, myocardium) were dissolved together and are called 'viscera'.

Fig. 1 shows that at all time-intervals the proportion of the total body radioactivity in the viscera (including liver) was greater in the depleted rats than in the normal rats; conversely, the activity in carcass and skin was less in the depleted than in the normal animals. This is not due to any difference in the gross distribution of protein. Table 1 shows that the distribution of total body nitrogen between liver, other viscera, carcass and skin was about the same in the two groups of rats. The only significant difference was in brain.

Table 2 shows the radioactivity of the tissues studied in relation to that of serum. In the protein-deficient animals the incorporation of labelled amino-acid was relatively much less in muscle and in skin. This finding may have some clinical relevance, since both in animals and in man changes in skin and hair are prominent when the diet is low in protein.

There are several possible explanations of this effect.

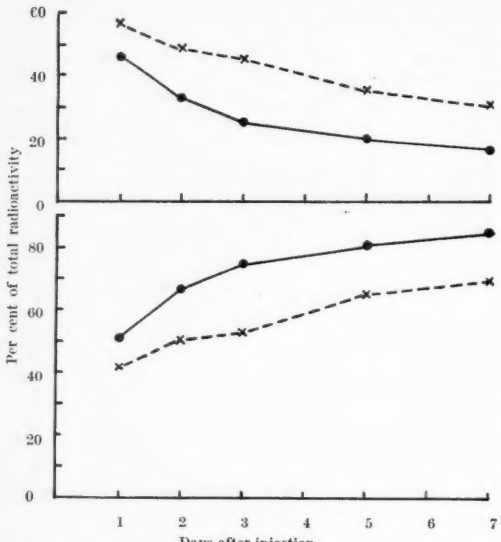

Fig. 1. Top, viscera (including liver); bottom, carcass and skin. ——, Normal; — — —, depleted

Table 1. Distribution of Body Nitrogen in Normal and Depleted Rats. (Figures are means ± S.E.M.)

	Normal	Depleted	Difference (*P*)
Number of rats	7	8	
Body weight (gm.)	141 ± 9·2	40·5 ± 5·4	
Per cent of total body N in:			
Liver	6·0 ± 0·37	5·9 ± 0·25	N.S.
Brain	0·72 ± 0·06	2·71 ± 0·31	0·001
Other viscera	9·3 ± 0·65	11·1 ± 0·56	N.S.
Carcass	57·1 ± 0·68	56·4 ± 1·03	N.S.
Skin	26·7 ± 0·99	23·8 ± 0·47	N.S.

N.S. = Not significant.

Table 2. Radioactivity (counts per mgm. Nitrogen) of Organs in Normal and Protein-Depleted Rats in Relation to that of Serum. (Activity of Serum taken as 100 in Each Animal)

	Days after injection	Liver	Brain	Other viscera	Muscle	Carcass	Skin
Normal	1	79	26	62	22	19	17
Depleted	1	81	27	79	21	21	13
Normal	2	69	31	61	25	27	26
Depleted	2	80	25	56	9	16	9
Normal	3	69	41	63	34	34	27
Depleted	3	73	29	57	17	19	11
Normal	5	61	35	56	44	53	40
Depleted	5	85	38	55	23	24	15
Normal	7	83	70	72	55	58	83
Depleted	7	100	63	75	47	49	22

(1) There may be a difference in the circulatory supply of building materials to the tissues, or in the rate of penetration of amino-acids into cells. Insulin, for example, increases both the rate of entry of amino-acids into muscle[4], and the rate of incorporation into muscle protein[5,6].

(2) Although there is no difference in the gross distribution of nitrogen, there may in the depleted animals be a selective loss from some tissues of protein fractions with a high turn-over rate. We found that in muscle sarcoplasmic proteins are reduced in the depleted rats, whereas collagen is relatively increased[3]. On the other hand, Luck[7] found no difference in the pattern of liver proteins in normal and protein-deficient rats.

(3) There may be true difference in the turn-over rate of tissue proteins. Because of re-utilization of the label, turn-over rates cannot be calculated from the present data.

(4) The results might apply only to experiments with methionine, which cannot necessarily be regarded as representative of all amino-acids. The changes found, particularly those in the skin, could be caused by a difference in the rate of conversion of methionine to cystine. Snyder, Cornatzer and Snyder[8] have shown that a low protein diet decreases the rate of disappearance of protein-bound methionine from the liver, but not that of cystine.

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HÆMATOLOGY

A Hæmoglobin with Unusual Alkaline-denaturation Properties in a Turkish-Cypriot Woman

In the course of investigation into thalassæmia in Cyprus, unusual features were discovered in the hæmoglobin pattern of the Turkish Cypriot mother of a boy of eight suffering from moderately severe thalassæmia. The hæmatological picture of the child was typical of this condition, and 60 per cent of the total hæmoglobin was of fœtal type (hæmoglobin F).

The child's father was dead. The mother presented clinical and hæmatological features of thalassæmia minor, and her red cells did not sickle. However, her hæmoglobin composition was quite unlike that normally associated with the thalassæmia trait. On paper electrophoresis in veronal buffer (*p*H 8·6, ionic strength 0·05) two hæmoglobin bands were apparent, one corresponding to hæmoglobin A (60 per cent of the total) and the other slower band (about 40 per cent) migrating behind hæmoglobin A in a position between those expected for hæmoglobins F and S (Fig. 1*a*). The hitherto described hæmoglobins which migrate on paper in this latter region are the Q[1], G[2], L[3] and 'Galveston'[4] variants. In agar electrophoresis[5] at *p*H 6·3 complete separation of two components was not achieved, but when compared with hæmoglobin A, a frontal zone of slightly higher mobility was frequently observed. In moving-boundary electrophoresis in phosphate buffer (*p*H 6·40, ionic strength 0·04) the abnormal hæmoglobin migrated with hæmoglobin A. The apparent mobility of the ascending composite boundary was less than that of hæmoglobin A (2·4 compared with $2{\cdot}6_3 \times 10^{-5}$ cm.^{2} V.$^{-1}$ sec. for hæmoglobin A) and its resolution from hæmoglobin A_2 correspondingly greater (Fig. 1*c*). Since hæmoglobin G can be resolved from hæmoglobin A under these conditions[6], this hæmoglobin is virtually excluded.

On the ion-exchange resin *IRC*–50 the abnormal hæmoglobin was eluted chromatographically at the same rate as hæmoglobin A by sodium citrate buffer (*p*H 6·1, (Na^+) 0·15 *M*). This behaviour distinguishes

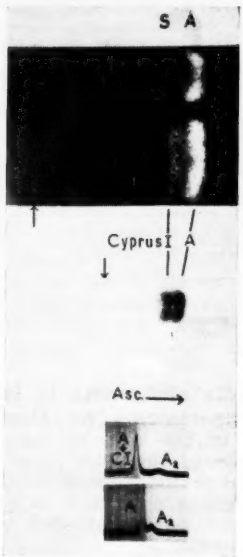

Fig. 1. (*a*), Paper electrophoresis of maternal red cell hæmolysate compared with sickle-cell trait in veronal buffer (*p*H 8·6, ionic strength 0·05). (*b*) Electrophoresis of maternal red cell hæmolysate on starch block in veronal buffer (*p*H 8·6, ionic strength 0·1). (*c*) Moving boundary electrophoresis (ascending limb) of maternal compared with normal adult red cell hæmolysate in phosphate buffer (*p*H 6·4, ionic strength 0·04)

it from hæmoglobins Q and L, and also G, leaving only 'Galveston' being possibly identical with the present hæmoglobin. Hæmoglobin A_2 was clearly visible in these columns, moving more slowly than the main band. The presence of the abnormal hæmoglobin did not cause any deviation in the solubility of the total reduced hæmoglobin of the hæmolysate from the normal figure, a value of 9·2/litre being obtained in 2·8 *M* phosphate buffer (*p*H 6·8, ionic strength 4·74) at 5° C.

The most striking property of the abnormal hæmoglobin, which would also appear to differentiate it from 'Galveston', was its behaviour on alkaline denaturation. On denaturation with *N*/25 sodium hydroxide, a rate plot indicative of two components was obtained, the faster-denaturing component being hæmoglobin A. The more slowly-denaturing hæmoglobin comprised about 40 per cent of the total, and had a half-reaction time of about 100 sec., compared[7] with 1,030 sec. for hæmoglobin F. The identity of the slowly-denaturing component with the abnormal electrophoretic component was further established by separating the latter by starch-block electrophoresis in veronal buffer (*p*H 8·6, ionic strength 0·1) (Fig. 1*b*) and elution; the half-reaction time for the slower zone was greater than that of the faster (hæmoglobin A) zone by a factor of about 3 (a more precise comparison was not possible because of low pigment concentration and partial conversion to methæmoglobin in the eluates).

The position of the tryptophan fine-structure band of the total hæmolysate was identical with that of hæmoglobin A, and its visible absorption spectrum was normal. The ¾-min. alkaline denaturation residue was high (15 per cent), but it contained no detectable hæmoglobin F. On the other hand, the blood of the child contained about 60 per cent of hæmoglobin F, estimated by both spectrographic and alkaline-denaturation rate methods[7]. This result, taken in conjunction with the appearance of a single band on paper electrophoresis in the position found for mixtures of hæmoglobin A and hæmoglobin F, suggests that the son does not possess the abnormal hæmoglobin.

The specific characteristics of the maternal blood were observed in three different samples, obtained over an interval of twelve months. It appears that the mother's red cells contain a hitherto unrecognized hæmoglobin variant, which although it does not resemble hæmoglobin F or fœtal-type hæmoglobin[8,9], differs from other adult hæmoglobins in its greater resistance to alkaline denaturation as well as in its electrophoretic behaviour. The genetic relationsip of this hæmoglobin variant, which we provisionally designate as hæmoglobin Cyprus I, to thalassæmia is not clear from the familial data at present available.

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Estimation of Small Percentages of Fœtal Hæmoglobin

In the alkali denaturation method of Singer, Chernoff and Singer[1] the one-minute residue of normal adult hæmoglobin gives a spectrophotometric reading of 0·5–1·8 per cent 'alkali resistant hæmoglobin'. It is well known[1,2,3], that most of this residue is not true fœtal hæmoglobin, but some other hæm-compound, the nature of which is unknown.

Studying the solubility of the denaturation product ('alkaline hæmatinglobin') in increasing concentrations of ammonium sulphate we found that it behaves like other proteins. The logarithm of the dissolved substance falls linearly with increasing salt concentration (Fig. 1). It can be seen, that at 33⅓ per cent saturation the precipitation of alkaline hæmatinglobin is not complete. Under the conditions of the experiment in Fig. 1 approximately 1 per cent of the alkaline hæmatin globin is still in solution.

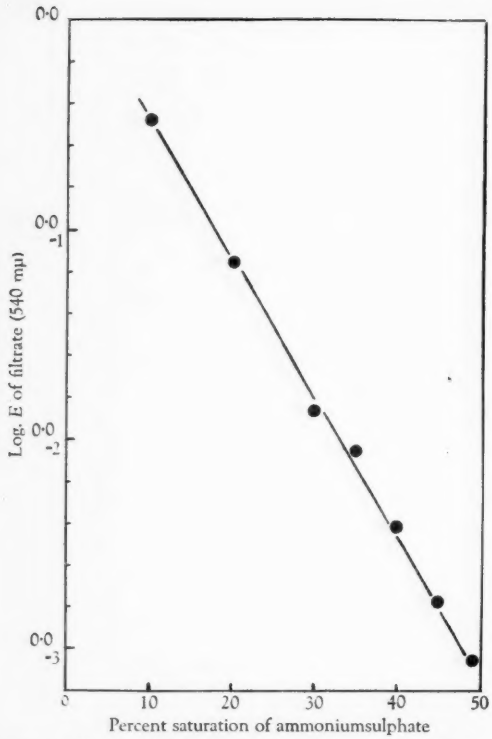

Fig. 1. Solubility of alkaline hæmatinglobin in increasing concentrations of ammonium sulphate. Hæmatinglobin produced by action of 0·08 *N* sodium hydroxide on 466 mgm. per cent cyanmethæmoglobin. Final concentration corresponds to 230 mgm. per cent

The post-denaturation reading can be diminished by raising the concentration of ammonium sulphate to 40 per cent. This concentration is still innocuous to the non-denatured fraction of hæmoglobin. A second point is, that adult hæmoglobin is not fully destroyed after 1 min. at 20°C. It is better to wait 2 min.

Considering these facts, the alkali denaturation procedure can be modified as follows: 2·8 ml. of a 500 mgm. per cent (450–600 mgm. per cent) solution of cyanmethæmoglobin are thoroughly mixed with 0·2 ml. of a 1·2 *N* sodium hydroxide. After 2 min., 2 ml. of saturated ammonium sulphate are added. The well-mixed content is filtered (twice through the same filter) after some minutes. The spectrophotometric reading of the filtrate at 540 mμ is compared with an adequate dilution of the original sample with water. In cases of high percentages of fœtal hæmoglobin the filtrate must be diluted (temperature 20–22°). It is not necessary to add hydrochloric acid to the ammonium sulphate. Oxyhæmoglobin is not as suitable as cyanmethæmoglobin; we found some irregularities in the denaturation rate as estimated with precipitation during the first 2 min. Furthermore the use of cyanmethæmoglobin avoids the possible error caused by CO-hæmoglobin accidentally present. The hæmoglobin solution must be prepared from washed red cells.

In 99 normal adults we found an arithmetic mean of 0·516 (±0·0119) per cent alkali-resistant spectrophotometric reading. Standard deviation was ±0·118, the total range extended from 0·24 to 0·75 per cent. From these results one can conclude, that values above 0·8 per cent are probably abnormal, while readings above 1·0 per cent are clearly so.

To what extent the post-denaturation residue may represent true fœtal hæmoglobin must still be clarified. Minimal amounts of cord hæmoglobin added to solutions of adult hæmoglobin gave values of hæmoglobin F as expected.

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Role of Stroma in Hæmoglobin Synthesis: Experiments on Duck Red Cells *in vitro*

It has been suggested that the formation of hæmoglobin takes place by the transformation of one protein (stroma) into another through an intermediate phase rich in non-protein nitrogen[1]. We have investigated this question using radioactive tracers.

Heparinized duck blood, with added sodium-benzyl penicillin and dihydrostreptomycin sulphate, was incubated for 90 minutes at 38·5°C. in an atmosphere of 95 per cent oxygen and 5 per cent carbon dioxide. 10 μc. of glycine-2-^{14}C (specific activity 1 mc./m.mole) and of 300 mgm. of glucose per 100 ml. of blood were also added. At the end of the incubation the red cells were separated by centrifugation and washed four times with saline containing 1 per cent of unlabelled glycine and four times with saline only. Washing was carried out at room temperature and continued for about 90 min.

The red cells were resuspended in unlabelled plasma taken from the same duck (1 vol. of red cells/1·5 vol. of plasma). Five samples were prepared and reincubated for 0, 1·5, 3, 6, 18 hours in the first run, and for 1, 2, 4, 8 hours in the second run. After incubation, the red cells were separated and washed four times with saline, and hæmolysed. The ghosts were separated by centrifugation; the supernatant was dialysed at 0°C. for a week against distilled water in a 5-l. vessel with a flux of 15 l. a day. The haem and globin were prepared from hæmoglobin in the usual way[2,3].

The ghosts were washed with saline and distilled water until the supernatant was free of hæmoglobin. The samples were analysed for their specific activity as previously described[4].

Table 1

Reincubation time (hr.)	Experiment No.	Globin α	Globin α*	Haem α	Haem α*	Ghosts α	Ghosts α*
0		185	1	3400	18·4	485	2·6
1·5		252	1	4890	19·4	471	1·9
3·0	1	277	1	5880	21·2	445	1·6
6·0		277	1	5815	21·0	530	1·9
18·0		285	1	5900	20·7	510	1·8
Mean value ± standard deviation				20·1±1·0		490±2·9	
0		97	1	2050	21·2	178	1·8
1·0		136	1	2750	20·2	210	1·5
2·0	2	141	1	2790	19·8	200	1·4
4·0		155	1	3020	19·5	190	1·2
8·0		152	1	3180	20·9	185	1·2
Mean value ± standard deviation				20·3±0·6		193±11	

α specific activity in disintegrations/min./mgm. carbon.
α* specific activity referred to globin specific activity at the same incubation time; error of experimental data <10 per cent.

The results are reported in Table 1. The specific activities of the globin, normalized to 280 disintegrations/mm./mgm. carbon for the first experiment and to 153 disintegrations/min./mgm. carbon for the second experiment, are shown in Fig. 1 plotted against reincubation time. In Fig. 1 the specific activities of the ghosts normalized to 490 disintegrations/min./mgm. carbon for the first experiment and 193 disintegrations/min./mgm. carbon for the second experiment are also plotted against reincubation time.

It can be seen that the hæm and globin specific activities attain their maximum values after 3–4 hours of reincubation, the increase is almost exponential (Fig. 1). The ghost specific activity on the other hand is constant within the limits of experimental error.

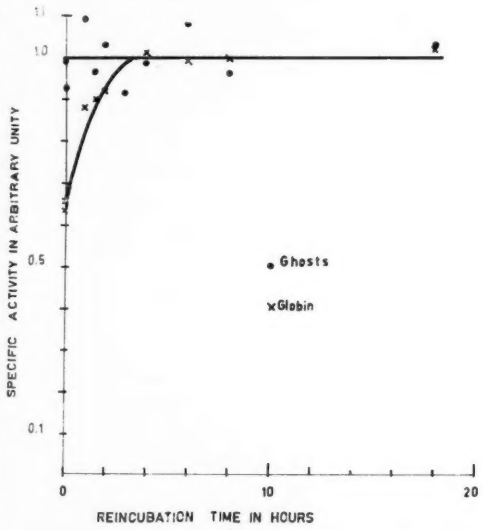

Fig. 1

We have already carried out the following experiment (in the press): duck blood was incubated with glycine-2-^{14}C as described above for 1·5, 3, 6 or 8 hours. The results obtained are reported in Table 2. We found that the activities of globin and ghosts increased during the whole incubation time, and that the ratios of specific activities of globin and ghosts were independent of the incubation time.

Our results do not support the suggestion about the role of stroma in hæmoglobin synthesis, because in the first set of experiments the increase of globin specific activity is not followed by a sufficient decrease of ghosts specific activity. They show that inside red cells there is a pool of radioactive substances, which cannot be removed completely by washing, and which is employed for hæmoglobin synthesis in the first 3–4 hours of reincubation. It is unlikely that there is a transport equilibrium of radioactive glycine from this pool inside the red cells, to ghosts and from them to globin, because the globin specific activity reaches its maximum value after only 3–4 hours of reincubation. After histine globin and ghost specific activities remain constant. Indeed the amount of the proteins synthetized, if they are not labelled, is too small to change the specific activities of the preformed proteins[5,6]. Furthermore, 3–4 hours are enough to remove completely the pools of radioactive precursors of hæmoglobin inside the red cells.

TABLE 2

Incubation time (hr.)	Experiment No.	Globin α	Globin α*	Ghosts α	Ghosts α*
1·5		254	1	336	1·3
3·0		425	1	543	1·3
6·0	1	688	1	622	1·3
18·0		765	1	812	1·1
3eMean value ± 3estandard deviation				1·2±0·1	
1·5		38	1	86	2·26
3·0		18	1	138	2·03
6·0	2	104	1	190	1·83
18·0		130	1	232	1·79
Mean value ± standard deviation				2·0±0·2	

α, α* and errors as in Table 1.

Finally it is surprising the ghosts do not take up the radioactivity inside the red cells, while, when radioactive glycine is added to the plasma, the ghosts take it up at a rate proportional to that with which hæmoglobin does so (Table 2).

Further experiments are in progress.

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Pathway of Carbon-^{14} Incorporation from $NaH^{14}CO_3$ to Hæm in Duck Blood incubated *in vitro*

Previously we have found that the hæm synthesized by duck blood in the presence of $NaH^{14}CO_3$ was radioactive[1] and that radioactive ketoacids, namely pyruvic and α-ketoglutaric acids were formed[2]. We suggested that Krebs' cycle was effective for the incorporation of carbon-14 into hæm via succinic acid. We have extended our experiments to prove this hypothesis.

Hæmin was obtained[1] by incubating 85 ml. of duck blood with 1·5 mc. of $NaH^{14}CO_3$ (specific activity 1 mc./mmole). The crude hæmin, about 200 mgm., was added to 1·4 gm. of inactive hæmin and crystallized[3]. The hæmin was degraded to hæmatinic acid and ethyl-methyl-maleimide according to Shemin *et al.*[4]. The specific activities of these compounds were measured by our usual method[1]. The results are given in Table 1.

It can be seen that the calculated activity of the methene bridges can be neglected as compared to the activity of mesoporphyrin. Furthermore, the specific

TABLE 1

Material	Specific activity (dis./min./mgm. C) Found	Calculated
Mesoporphyrin	16·4±0·6	—
Hematinic acid*	22·4±0·2	—
Methyl-ethyl-maleimide*	12·4±0·2	—
Carboxyl group of hematinic acid	—	92±4
Methenic bridge	—	6·4±7·2

* Purity tested by centesimal analysis and melting point.

activity of the methene bridges is of little significance, because of the experimental error.

The results suggest that the carbons of pyrrole derivatives derived from the carboxyl groups of succinic acid are not labelled uniformly. Indeed, the carboxyl group of hæmatinic acid has half the radioactivity of this compound.

Shemin *et al.* incubated duck blood with succinic acid labelled at its carboxyl groups: they found that the carbon atoms 3, 5, and 10 of the porphyrin molecule (Fig. 1) had the same specific activity. With $CH_3{}^{14}COOH$, they found almost all the incorporated radioactivity in carbon atom 10. They explained these results by suggesting the existence of an asymmetric derivative of succinic acid in hæm synthesis[5]. Our results can also be explained in this way.

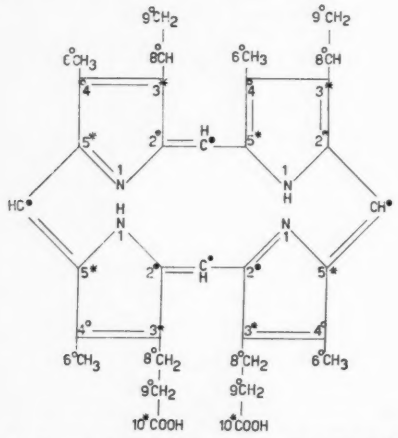

Fig. 1. Sources of porphyrin carbon atoms according to Shemin. *, from the carboxyl group of succinic acid; ●, from the α carbon atom of glycine; ○, from carbon atoms 2,3 of succinic acid

In order to test whether the radioactivity of succinic acid, employed in hæm synthesis, comes from α-ketoglutaric acid, we partially degraded this acid. The α-ketoglutaric acid was isolated from the plasma together with pyruvic acid. The ketoacids as 2–4 dinitro-phenyl-hydrazones were separated by paper chromatography[2]. The 2–4 dinitrophenylhydrazones of pyruvic acid and of α-ketoglutaric acid were diluted with about 300 mgm. of each inactive synthetic hydrazone to obtain an adequate bulk of material.

The 2–4 dinitrophenylhydrazone of α-ketoglutaric acid was assayed for its mean specific activity and for its carboxyl specific activity in the following way. The acid in 3*N* sodium hydroxide was decarboxylated adding a saturated solution of potassium hydroxide to which 50 per cent sulphuric acid had been added until the solution was 1*N* with respect to the acid. The decarboxylation was carried out in a Van Slyke apparatus. We found that the amount of carbon dioxide evolved was lower than that reported by Krebs *et al.*[6]. This agrees with the results of Shemin *et al.*[5].

The specific activity of the 1-carboxyl group of α-ketoglutaric acid was determined from the total activity of the carbon dioxide evolved and the amount of 2–4 dinitrophenylhydrazone employed for each experiment, assuming that only the 1-carboxyl group is degraded by this method. Succinic acid was extracted from the residual solution with ethyl ether and assayed for its specific radioactivity. The results are reported in Table 2.

Table 2

Material	Specific activity (dis./min./mgm. C) Found	Calculated
2–4 d.n.p. of α-ketoglutaric acid*	112±4	—
α-Ketoglutaric acid	—	246±8
Carboxyl group of α-ketoglutaric acid	1168±240	1206±42
Succinic acid from decarboxylation of α-ketoglutaric acid*	6·0±0·4	—
2–4 d.n.p. of pyruvic acid*	40±2	—
Pyruvic acid	—	121±6
Carboxyl group of pyruvic acid	204±40	—
Carbon atoms 2–3 of pyruvic acid	—	156±58

d.n.p. = dinitro-phenylhydrazone.
* Purity tested by centesimal analysis and melting point after the dilution.

From these results and from the amount of hæm synthesized *in vitro*[1] we can argue that the radioactivity of succinic acid, employed in hæm synthesis, cannot come from the decarboxylation of α-ketoglutaric acid because almost all the radioactivity of the acid is in the 1-carboxyl group. We can conclude that radioactive succinic acid occurs via the dicarboxylic acid cycle: pyruvic $\xrightarrow{CO_2}$ oxalacetic ⟶ malic ⟶ ⟶ fumaric ⟶ succinic.

In order to test whether the carbon atoms of the porphyrin molecule coming from carbon atoms 2 and/or 3 of succinic acid are also labelled, we have partially degraded pyruvic acid.

The 2–4 dinitrophenylhydrazone of pyruvic acid was decarboxylated in two ways.

(1) By reductive decarboxylation with stannous chloride in acetone, acidified for concentrated hydrochloric acid. The hydrazone (58 mgm.) was refluxed in a three-necked flask connected with a Van Slyke apparatus under vacuum with 50 ml. of acetone containing 2 ml. of concentrated hydrochloric acid for 4½ hr. The solution was cooled and 1 gm. of stannous chloride in 4 ml. of concentrated hydrochloric acid was added. The mixture was again refluxed for 2 hr. The yield of the decarboxylation reaction was lower than theoretical.

(2) By oxidative decarboxylation with $Ce(SO_4)_2$ in 10 *N* sulphuric acid[8]. In this case as well the yield was lower than theoretical.

The results are reported in Table 2. We found carbon atoms 2 and/or 3 of pyruvic acid labelled.

That carbon atoms 2 and/or 3 of pyruvic acid are labelled suggests that pyruvic acid is formed by some other route besides the dicarboxylic acid cycle.

We therefore believe that carbon-14 fixation from $NaH^{14}CO_3$ in hæm of duck blood *in vitro* takes place via the dicarboxylic acid cycle and that carbon dioxide is not utilized directly to link the pyrrole derivatives in the porphyrin molecule.

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RADIOBIOLOGY

Effects of the Energy of Strong Pulses of Ionic Current on Micro-Organisms in Suspension

In a general survey of the lethal effects on micro-organisms of passing extremely strong but very brief pulses of current through solutions suspending them comparable attention has been given both to the biological effects and to the physical actions.

The suspensions, usually in quarter-strength Ringer's solution +1 per cent glucose, but sometimes in phosphate buffer, milk, yeast-dextrose broth or ale, were made to flow rapidly between immersed electrodes of platinum or tungsten. In an arbitrary standard treatment used in most of the biological comparisons the 30 pulses were each of 0·6 μsec. duration and had current densities of 125–150 amp. per cm.^{2}, field strengths 30–40 kV. per cm., thus dissipating energy at rates of 4·5–5·5 MW. per c.c. Other weaker prolonged treatments revealed that, unlike some other physical treatments, these current pulses have a threshold, generally of the order of 30 amp./cm.^{2}, 7 kV./cm., and 0·2 MW./c.c. which was only just reached by Nyrop[1] using condenser discharges. The highest power-density used was 9·3 MW./c.c.

The standard treatment was applied to suspensions, initially at room temperature, of 20 bacteria, 3 of them anaerobic, 4 microaerophilic, 9 facultative and 4 aerobic, 3 yeasts, 1 mould (spores), and 1 bacteriophage. As between the classes of bacteria the mortalities were in the inverse order of tolerance of oxygen, for example, from 99 per cent for *Clostridium butyricum*, to 90 per cent for *Escherichia coli*, 27 per cent for *Bacillus mycoides*, and only 6 per cent (increased to 50 per cent in the survivors from a previous ultra-violet light treatment) for *Micrococcus candidus*, ; the factor of inactivation of 'phage' was 10^8.

On the acid-fast waxy-coated *Mycobacterium smegmatis* there was no effect. There was no correlation with the Gram-stain property, nor with the responses to ultra-violet light, which differed little among ten of the bacteria including *M. smegmatis*. As regards dosage there is a general rule, exemplified by six organisms, including the mould spores, that the survival is inversely proportional to the dose by numbers of similar pulses. There are a few interesting exceptions: in particular with cultures of *E. coli* under a critical degree of aeration repeated treatments with interposed subcultures of survivors show a progressive decrease of susceptibility which is at least transitorily heritable in subsequent subcultures. This is the less likely to be due to extensive initial inhomogeneity since the effect is the same for a single-colony isolation as for stock cultures, but may represent heritable changes in individual survivors. *M. candidus* behaves similarly, but fully aerated.

The comparisons between bacteria suggested that the treatment was basically oxidative: variations in the environmental conditions confirmed this. Dissolved oxygen was a contributory factor, for in deaerated solution under argon the mortality of *E. coli* fell to 50 per cent, a diminution comparable with that reported by Dewey and Boag[2] for *Serratia marcescens* irradiated with X-rays or electrons; with *M. candidus* it fell to zero. Further, 0·2 per cent sodium thioglycollate present in the solution protected *E. coli* almost completely, and phage notably, and if added even as much as 10 min. after treatment partially revived *E. coli*, *Streptococcus lactis*, and phage; anaerobes, however, gave their response in presence of the S.T.G. Treated solutions containing no organisms but tinted with methylene blue developed transitory colour corresponding to as much as 0·4 mgm./per ml. of additional oxygen, but did not do so when de-aerated under argon. In the case of the cytochrome-bearing obligate ærobe *M. candidus* not only is oxygen essential, but the mortality is increased to 96 per cent if the suspension is initially chilled to about 4°C., an expedient successfully adopted by my former colleague M. H. Mannering in view of Keilin's[3] observation of the failure of a yeast to reduce cytochrome in chilled solution. This effect increases rapidly below 7°C., but in de-aerated solution at 4°C. there was no mortality. A double-length treatment at 9·3 MW./c.c., left a survival of only 1 in 10^6. With *E. coli* in the cold there was a rapidly increasing susceptibility as though by a cumulative effect in the last half of a double standard treatment leading to 1 in 10^5 survivals, or to 1 in 10^6 at 7 MW./c.c. This effect had a threshold at 3·8 MW./c.c., but de-aeration suppressed it. Both *M. candidus* and *E. coli* could be partially revived by the addition after standard treatment of methylene blue which can perform the functions of inactivated cytochrome.

Coming now to the chemical and physical aspects, in the first place the electrolytes needed to carry the current would yield decomposition products, but the evidence about these as responsible agents is adverse, for example, chlorine from the usual Ringer might be held dangerous, but the effect is unchanged if phosphate buffer of any *p*H between 5 and 8 is substituted: in de-aerated Ringer in the cold *M. candidus* is quite unaffected: physically again, there is no correlation between the effect and variations in the electrical conditions in respect of the quantities of electricity passed. Secondly, there is very appreciable heating, 0·75°C. per standard 0·6 μsec. pulse owing to the high rate of dissipation of energy, and indeed it was usual to interrupt the treatment for intermediate cooling limiting the maximum temperature to only 30°C. However, the pulse effect was unchanged for *E. coli* when the suspension had been previously heated to 40°C., then rising to 52°C.; conversely, the small effect on the heat-sensitive *M. candidus* was increased by chilling. Comparable heating by other methods had negligible effects. Further, when the temperature rises had been restricted to 2 or 3°C. in weaker but longer treatments the effects of the standard treatment were reproduced. Such small heating should be biologically insignificant, and on this and the other evidence cited it was concluded that the action was not thermal.

Comparison of results using solutions of different concentrations showed that neither the current density alone nor the field strength alone was a sufficient determining factor; it was their product, the power-density, that decided all results. This fact ruled out actions dependent on the field alone, such as the transient currents induced within the organisms only during the rise and fall of a pulse, or the induced charges built up by them and persisting during the pulse maximum on either side of the cell wall[4,5]. Electronmicrographs showed no disruption nor visible internal changes.

On combining with their power-density the total duration of the pulse maxima in treatments of various power-densities, which gives the total dissipation of energy, it transpired that the effect was the same for equal energies over a range of power-density between 1 and 4 MW./c.c. for the representative micro-organisms *Cl. butyricum*, *E. coli*, *Str. lactis*, two yeasts, and the 'phage virus whatever the variations in

pulse-length, 0·6–50 μsec., or rate of recurrence, 100–500 per sec., power-density, maximum rise of temperature, 14–2°C., or electrolyte concentration.

These results posed the main physical problem of relating energy in the body of the solution, expressible as total calories per c.c., with the localized effect on the micro-organisms, taking into account that major effects could be produced with a rise of 2 or 3°C. Now it can be shown that the energy expended in the double-layer adjacent to the organism and that in the body of the solution are both proportional to (ionic drift velocity)^2 × concentration. The localized action can be further correlated with that measured in the solution by comparing linear separations between ions in both regions[6]; these prove to be so nearly the same, about 20A., that the two energies could well be of the same order, whereas for selective heating of so small a body as a cell the local energy would have to be enormously the greater. Hence the idea arises of ascribing the primary physical action to sufficient ionic disturbances in the double-layer. This idea is supported by the reproductions of the main pulse effects observed after another kind of ionic disturbance caused by change of the ionic environment when organisms are centrifuged from one solution and re-suspended in another[7], and more strongly by the fact that when the pulse treatment is preceded by such an ionic change its effect is much modified.

The pulse action may thus be considered as an orderly march of the cations of the double layer over the surface of the organism, superposed on their random motions, and as successive ions pass a point producing disturbances at intervals of the order of 10^{-8} sec. which are comparable with the relaxation times of water molecules in the neighbourhood of ions[8]. To such disturbances, unusual in number or kind, the organisms are not apparently adapted, and viability fails; a single 0·6 μsec. pulse can so kill some 7 per cent of *E. coli* or 17 per cent of *Cl. butyricum*. Further, because of the very short time intervals involved, it can be supposed that the disturbances take effect biologically before the equipartition of energy in subsequent collisions degenerates their energy into random motions recognizable as heat. That such disturbances are capable of affecting sensitive chemical equilibria is shown by the displacement of the equilibrium between methylene blue and oxygen.

A detailed account of this work has been prepared for publication.

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Biological Half-Life of Radioiron in Man

The recommendations of the International Commission for Radiological Protection, 1955[1], give in Table *C*.V. values of the effective half-life for iron-55 and iron-59 of 61 and 27 days respectively. The fact that these values are so similar in spite of the big difference in their physical half-lives ($1·06 \times 10^3$ and 46 days respectively) results from the use of a biological half-life for iron in the blood and blood-forming organs of 65 days (Table *C*.IV). This result is probably based on early data obtained with animals (for example, Greenberg and Copp[2]) and would now appear to be considerably too low for the adult man.

An increase in the value of the biological half-life would not affect the calculation of the maximum permissible body burden, but would lead to a decrease in the maximum permissible levels in water and air, these being inversely proportional to the effective half-life in the conditions of final equilibrium with chronic exposure as considered by the Commission.

Under these equilibrium conditions one can assume that the body store in the critical organ is constant and the daily excretion and ingestion are equal. Then the biological half-life T_b may be calculated (as in their equation *C*4) from:

$$T_b = \frac{0·693 \text{ (mass of element in initial organ)}}{\text{daily uptake into (or excretion from) critical organ}}$$

Table *C*.II gives the mass of blood as 5,400 gm., containing 5×10^{-4} gm. iron per gm. blood, and the uptake as 80 per cent of a dietary daily intake of 12 mgm. of iron. Using these figures in the above equation leads to a biological half-life of 195 days—already some three times the value used in the calculations.

However, recent absorption studies[3,4] indicate that not more than 10 per cent of the dietary iron is, in fact, absorbed in the normal adult.

Further, although iron is released after red cell destruction, it is diluted in body pools and re-utilized for further red cell production, so that the net excretion loss is very small indeed. Callender[5] states that the adult male has about 2–3 gm. of iron in hæmoglobin and 1–1·5 gm. in body stores (ferritin and hæmosiderin). This total is somewhat higher than the 2·7 gm. of iron in the critical organ given by the Commission's data. The excretion of iron in the fæces does not exceed 0·5 mgm./day[6], the loss from all sources being only about 1·5 mgm./day, corresponding to some 10 per cent (that is, the absorbed fraction) of the dietary daily intake of 10–15 mgm. Then, using values of 1·5 mgm. daily loss and a store of 3 gm. leads to a biological half-life of 1,400 days, corresponding to effective half-lives of 600 days for iron-55 and 44·5 days for iron-59, increases of 10 times and 1·6 times, respectively, over the present figures given by the Commission.

It should be appreciated that in childhood the iron stores are in the process of being built up and during this period iron uptake will always exceed the excretion. Thus, equilibrium conditions are not achieved and the effective half-life will approximate to the physical half-life.

That such discrepancies should exist in data, which are widely quoted and accepted as standard, emphasizes the need for continual review of such tables, and for the need for workers to pass on to the respective International Commissions any new experimental results which may have a bearing on their calculations.

Note added in proof.
We have been informed by Dr. K. Z. Morgan, chairman of Committee II of I.C.R.P., that corrections of this type have already been incorporated in the revised recommendations which are being prepared.

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Cells which Synthesize Deoxyribonucleic Acid in Guinea Pig Bone Marrow During Recovery from Whole-Body γ-Irradiation

HARRIS's experiments[1,2] have shown that about the 14th day after whole-body γ-irradiation of young male guinea pigs the marrow is considerable simplified in the types of cells which it contains. At this time an accumulation of round cells was noted in the marrow. This accumulation preceded a rapid regeneration of proerythroblasts and myeloblasts. The round cells were of two types: (1) small cells which, morphologically, closely resemble small lymphocytes in smear preparations of lymphoid tissue; (2) cells intermediate in appearance and size between small lymphocytes and blast cells. A characteristic of the intermediate cells is the high ratio between nucleus and cytoplasm. On staining with MacNeal's tetrachrome, the nuclear chromatin has a relatively uniform appearance. In some cells the cytoplasm appears to be minimal whilst in others it is located in a well-defined nuclear hof. Apart from their morphology, certain findings in these experiments suggested the possibility that the intermediate cells might be considered as early free stem-cells. These are: (1) their quantitative relation to blast cells in the marrow of control animals; their total number is about 20 per cent less than the combined numbers of proerythroblasts and myeloblasts, and conforms to the quantitative finding in normal marrow that the more immature cells are comparatively few in number; (2) quantitative studies show that these cells are even more radiosensitive than myeloblasts and proerythroblasts; (3) mitotic figures were seen in cells of their size, shape and cytoplasmic content; and (4) considerable numbers of these cells preceded a rapid regeneration of myeloblasts and proerythroblasts.

Mitosis is a property of the more immature forms of marrow cells. Lajtha *et al.*[3] have investigated the synthesis of deoxyribonucleic acid which precedes mitosis in marrow cells. Since tritium-labelled thymidine is incorporated into deoxyribonucleic acid it may be used to label cells which are capable of the synthesis of this acid. Studies have therefore been made for evidence of synthesis of deoxyribonucleic acid in bone marrow cells of guinea pigs about the 14th day after 150 r. whole-body γ-irradiation.

Each animal was irradiated under general anæsthesia (intra-peritoneal sodium pentobarbitone), the source of irradiation being cobalt-60, average energy 1·2 MeV. The source to skin distance was 60 cm., dose-rate 70 rads/min. The field size was such as to include a reasonable margin of irradiation around the profile of the whole animal. The animal was backed with a 'Pressdwood' phantom so that normal depth dose curves could be applied with confidence. On the 14th day after irradiation, tritium-labelled thymidine (specific activity 61 mc./m*M*) was either injected in a dosage of 1 μc. per gm. body-weight, or a marrow suspension prepared in fresh autologous serum and the cells incubated with the label at 37° C. for periods varying from 30 min. to 1½ hr., based on the technique described by Bond *et al.*[4] Marrow smears were prepared 30 min.–1½ hr. after exposure of the marrow to the label. Smears were fixed in methyl alcohol and coated with fine-grain stripping film (Kodak *A.R.* 10) using the technique described by Pelc and Glucksman[5]. After 5–6 weeks exposure, the films were developed and the smears stained through the film with 0·25 per cent methylene blue at a controlled *p*H. Since it was desired to exclude the possibility of labelling of daughter cells, the marrow was not exposed to the label for longer than 1½ hr. The studies of Lajtha *et al.*[3] have shown that an interval of about 4 hr. separates completion of synthesis of deoxyribonucleic acid from the onset of mitosis in human marrow cells in culture. No evidence of labelling was seen in mitotic figures in smears examined from the present experiments. Preliminary studies have been made in 6 animals and the findings in a larger series of animals will be reported elsewhere.

Particular attention has been given to evidence of labelling in the cells which are intermediate in appearance between small lymphocytes and blast cells, and for evidence of labelling in the small round cells which have been termed small lymphocytes. Labelling was seen in cells which were classified as: (1) myeloblasts; (2) erythroblasts; (3) 'ghost' cells which appeared to be damaged cells in which distinctive features were not recognizable; (4) cells intermediate in appearance between small lymphocytes and blast cells (Fig. 1).

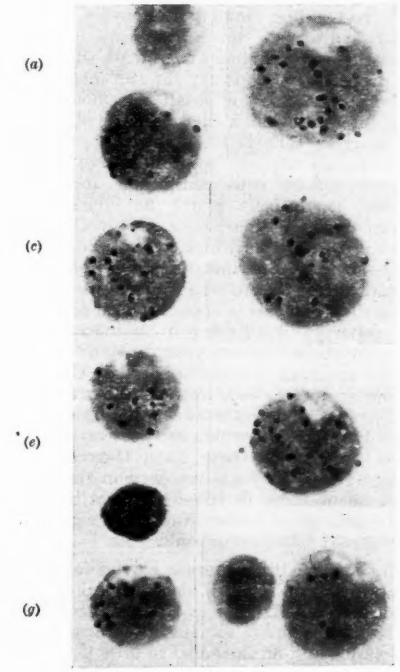

Fig. 1. Intermediate forms of cells in smear preparations of guinea pig bone marrow, 14 days after whole-body γ-irradiation. Labelling with tritium-labelled thymidine 30 min.–1½ hr. after exposure to label. Note the high ratio of nucleus to cytoplasm and the small amount of cytoplasm located in a well-defined nuclear hof. Cytoplasm in cell *d* appears to be minimal. The small unlabelled cells in *a* and *h* are small lymphocytes, and *e* also shows the nucleus of an advanced form of erythroid cell. Autoradiographs stained with methylene blue (× *c.* 1330)

The frequency of labelling in these intermediate cells was striking. In the smears examined there were many small round cells which showed no evidence of labelling. These cells have been termed small lymphocytes, and the absence of labelling in such cells supports this classification. Thus, Bond *et al.*[4] found

no evidence of synthesis of deoxyribonucleic acid in small marrow lymphocytes. Gowans[6] found minimal evidence of labelling in small lymphocytes of thoracic duct lymph, even after prolonged administration of labelled thymidine. In the experiments of Yoffey *et al.*[7] after injection of labelled thymidine, labelling was initially detected only in the larger cells of thoracic duct lymph. Labelling in substantial numbers of small lymphocytes was not seen until about the 4th hr. In view of the length of the interval before labelling of small lymphocytes was apparent, and from the observations of Lajtha *et al.*[3], it seems possible that the labelled small lymphocytes in the experiments of Yoffey *et al.*[7] were daughter cells resulting from mitosis of labelled precursors.

These experiments show that the round cells which accumulate in irradiated guinea pig bone marrow before the final regeneration of substantial numbers of granulocytes and erythroid cells, may be divided into two groups according to their ability to synthesize deoxyribonucleic acid: (1) cells intermediate in appearance between small lymphocytes and blast cells appear to be capable of synthesis; (2) many small round cells, resembling small lymphocytes, show no evidence of synthesis. Since synthesis of deoxyribonucleic acid is a preliminary to mitosis, the findings support a proliferative capacity for the intermediate forms of cells, but not for small lymphocytes. In addition to differentiation, a characteristic of the more immature marrow cells is an ability to proliferate. These experiments support a proliferative capacity for the intermediate cells and their morphology suggests that these cells might be considered as precursors of definitive blast cells. If small lymphocytes transform into cells which are morphologically intermediate between lymphocytes and blast cells, then they must change from cells which appear to show no evidence of proliferative capacity into cells which have this property. The finding in these experiments of evidence for the proliferative capacity of the intermediate cells suggests that, as opposed to the formation of the intermediate cells from small lymphocytes, the reverse may occur. In the event that at least some of the small lymphocytes which are present in irradiated marrow are formed there, then Harris's experiments[2] suggest that they are retained in the marrow where they accumulate in large numbers before the regeneration of cells of known functional significance, that is, erythroid cells and granulocytes.

We wish to thank Prof. Francis Davies for research facilities and advice. We are also grateful to Dr. H. Miller and Messrs. H. Fry and B. Watson of the Sheffield National Centre for Radiotherapy for their ready assistance and advice; also to Miss C. J. Crockford for skilled technical assistance.

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Measurement of the Rate of Growth of Wool using Cystine labelled with Sulphur-35

When cystine labelled with sulphur-35 is injected intraperitoneally[1] or intravenously into a sheep, radioactivity is detectable in the wool follicles, just above the bulb, within a few minutes, and appears in the fully keratinized fibre some hours later. If the rate and position of entry of cystine into the fibres can be presumed to be constant, repeated injections of labelled cystine at known intervals of time followed by autoradiography of the fibres should provide a method of measuring wool growth. Paduceva[2] has discussed this possibility without, however, giving any quantitative results to assess the accuracy of the method.

An opportunity to determine the accuracy arose during a study of cystine metabolism in the sheep. Cystine labelled with sulphur-35 DL-cystine (45 μc.; 0·25 mgm.) was injected intravenously into a Corriedale ewe (*c.* 50 kgm.). Eight days later the animal was given a second dose (349 μc.; 2·12 mgm.) and three days later still a third dose (348 μc.; 2·16 mgm.). For the first dose the cystine, as the hydrochloride, had been mixed for several days with plasma from the same animal so that most of the labelled cystine was bound to the plasma proteins whereas the other two doses comprised free cystine hydrochloride in 0·9 per cent sodium chloride solution. Although the three doses were not identical, an examination of blood samples taken at intervals after each injection showed that any difference in the time required for the two forms of cystine to reach the wool follicles was negligible. Just before the first injection the wool was clipped as evenly as possible from defined areas on the mid-side. Ten days after the third dose, samples of wool were carefully clipped from these areas to provide fibres representing 21 days growth and containing three radioactive 'spots'. A week later several more samples (28 days growth) were collected.

Each sample of fibres was washed well with ether, alcohol and water, allowed to dry, and mounted on microscope slides. The amount of adhesive (Mayer's egg albumen) used ($<0{\cdot}1$ mgm./cm.^{2} when dry) caused negligible absorption of the β-particles. About twenty separated fibres could be mounted on each slide. The samples were then autoradiographed (Kodak *A.R.* 50 film) using the 'stripping-film' technique described by Doniach and Pelc[3]. After 14 days' exposure some of the films were developed. This exposure time gave satisfactory autoradiographs corresponding to the second and third doses but longer exposures (about 6 weeks) were needed to make detection of the first dose easy. Fig. 1, shows a typical autoradiograph.

To measure the rate of growth of the fibres, the slides were examined with a projection microscope ($\times 215$) and the distances between the points of increasing silver grain density (as shown in Fig. 1) were measured with a flexible rule. The mean growth rates of 10 fibres measured independently by four different observers differed by less than ± 2 per cent. When each observer measured the same fibre more than once greater accuracy was obtained. However, since the variance between observers was found to be negligible compared with the variance between fibres, only two measurements, one for each growth period, were made on each fibre.

The mean growth-rates for the two periods of 8 and 3 days were about the same. Since the sheep

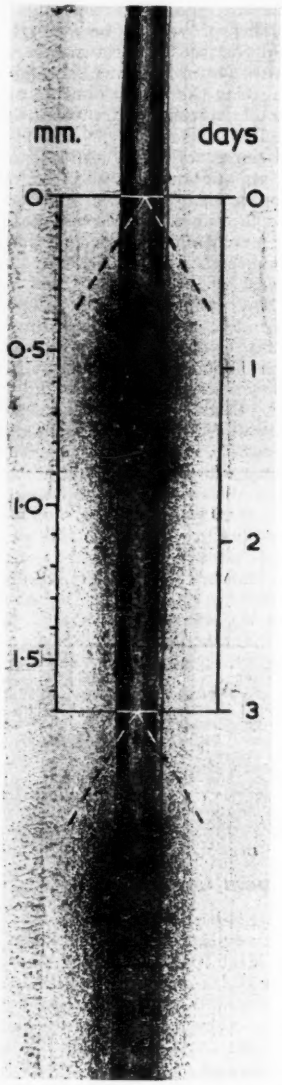

Fig. 1. Photomicrograph of a sheep vibrissa and overlying autoradiograph showing the method of measuring the length of wool grown during the three-day period referred to in the text. To measure this length, reproducible reference points were needed. It was necessary to find the point on the fibre corresponding to the time of each injection. This was determined, approximately, as the point of intersection of the lines drawn by eye (as shown) along the outer limits of each darkened region. In practice this region is not sharply defined so that different observers do not draw the lines in exactly the same positions. Nevertheless four observers, using their own criteria, produced results in good agreement (see text).

had been maintained at a constant nutritional level during the experiment and for several months before, it was assumed that the growth-rate was constant throughout the experimental period of 28 days. Based on this assumption, the autoradiographic method could be compared with a conventional method of estimating wool growth. Some of the fibres which had grown over periods of 21 and 28 days (including the 11 days covered by the autoradiographic method) were measured by the two methods. The results, shown in Table 1, were in agreement within the order of accuracy of the measurements; the autoradiographic method, based on 8 or 3 days growth, was, if anything, more precise than the conventional method based on 3 or 4 weeks growth.

Table 1. GROWTH-RATES OF WOOL FIBRES MEASURED BY AUTORADIOGRAPHY AND A CONVENTIONAL METHOD*

Method	Growth period (days)	No. of fibres	Mean growth rate (mm./day)	Standard error of the mean
Autoradiographic	8	168	0·511	±0·0083
	3	195	0·499	±0·0079
Conventional	21	160	0·544	±0·0118
	28	160	0·500	±0·0100

* Using a projection microscope (×43·5) the total lengths of the fibres were measured with a tracing wheel attached to a revolution counter.

The autoradiographic method of estimating growth of wool has two main advantages: the growth of wool fibres can be measured with good accuracy over short periods, of the order of a few days; and repeated measurements of growth-rates can be made without having to remove the fibres from the sheep until the end of the experiment, thus avoiding possible side effects due to frequent shearing or shaving.

These advantages should make possible accurate studies of climatic, nutritional or other effects on wool growth over short periods. The autoradiographic technique may also provide another method of measuring the percentage of active follicles and of studying the shedding of fibres in sheep and presumably also in other mammals.

Applications of this autoradiographic technique along the lines suggested are in progress at this laboratory and will be reported later.

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BIOLOGY

Biological Properties of Coal and Coal Extracts

Evans[1,2], as a result of studies on the cause of coalminers' pneumoconiosis, discovered that methanolic extracts of certain coals exerted an inhibitory effect on bacteria *in vitro*. He named the component responsible for this inhibition 'vitricin', from its apparent association with vitrain—the bright, vitreous ingredient of banded bituminous coal. Evans questioned the assumption that inhaled coal particles are biologically inert, and suggested that dusts derived from bacteriologically active coals might exert a prophylactic effect in the lung.

Kosanke[3] confirmed the extraction of bacteriostatic substances from lignite, sub-bituminous and high-volatile bituminous coals by methanol, and also showed that acetone and ethanol were effective solvents. These extracts were assayed for anti-fungal activity by Schenck and Carter[4], and found to inhibit the germination of spores of a variety of saprophytic and plant-pathogenic fungi.

It has since been shown that certain coals contain several substances inhibiting the growth of micro-organisms. For brevity, the name 'vitricin' is now applied to the group of biologically active substances contained in coal. A summary of some results of further research directed towards the isolation and characterisation of vitricin[5] follows; a detailed account will be published elsewhere.

Attempts to extract vitricin from coals with water, aqueous acids, alkalis, buffer solutions and aqueous-organic mixtures proved unsuccessful. Bovine serum did not acquire inhibitory properties when powdered coal was steeped in it; and coal did not inhibit the growth of bacteria when placed upon inoculated nutrient agar. Inhibitory extracts were obtained only with organic solvents, acetone being most often employed.

Considerable difficulty was experienced with the microbiological assay of these crude, tarry extracts. The technique finally adopted was a modification of the filter paper disk method of Vincent and Vincent[6], the powdered coals being extracted directly into small paper disks. These impregnated disks were placed upon the surface of inoculated nutrient agar, and, after incubation, disks containing active material gave rise to circular zones of inhibition. The standard test organism was *Bacillus subtilis* N.C.T.C. 8236, although tests were also made with other bacteria and moulds.

A systematic study was made upon a series of coals of increasing rank to find the most active coal and the optimum conditions for its extraction. Activity appeared greatest in the high-volatile bituminous coals, being slight or absent on either side of this range. It has been demonstrated that inhibitory properties are not associated with oxidation products of coal formed during grinding, storage or testing.

Coal from the Tupton seam, Glapwell Colliery (Notts.-Derby coalfield), of rank 701 in the National Coal Board Coal Classification System, was selected as a suitable source material. Maximum yield of vitricin was obtained by stirring the finely-powdered coal at room temperature with several successive changes of acetone and combining the filtered extracts.

This crude extract proved bacteriostatic towards both Gram-positive and Gram-negative bacteria, although the former were more sensitive. *B. subtilis* was strongly inhibited, serial dilution tests showing the minimum concentration of solute required to produce bacteriostasis to be 30 μgm./ml. in plain nutrient broth, and 90 μgm./ml. in the presence of 10 per cent v/v sterile bovine serum. The plant pathogens *Corynebacterium fascians* and *Agrobacterium tumefaciens* were also much affected, and activity against saprophytic and plant-pathogenic fungi has been confirmed.

Acidic, basic, phenolic and neutral components were present in the crude extracts, the last in much the greatest proportion. Attempts to separate active components by extraction of bacteriologically-active ethereal extracts with aqueous acids, alkalis and buffer solutions have so far proved disappointing, little activity being transferred to the aqueous phases.

Determination of the thermostability of a crude extract showed that it retained activity after heating to 100°C. for 1 hr.; but degradation leading to complete loss of activity was observed above 120°C. This behaviour suggested steam-distillation as a possible method of purification, and treatment of a concentrated acetone extract of Tupton coal with steam at 100°C. gave a 0·24 per cent w/w yield (based on weight of coal originally taken) of a pale yellow, limpid oil. This proved to be strongly inhibitory towards Gram-positive bacteria and certain moulds, but the fact that Gram-negative bacteria were almost unaffected suggests that additional factors, probably of a more labile nature, are present in the crude extracts of coal.

Direct treatment of coal with steam at 100°C. removed no organic matter, and the coal retained inhibitory properties. Steam superheated to 120°C. removed a trace of blue-fluorescent material, but at about 150°C. evolution of hydrogen sulphide began and a yellow-brown oil co-distilled. This oil was bacteriologically active, but the yield was so small (0·02 per cent) that no further investigation of this process was made. However, according to Ahmad[7], a similar liquid obtained as a by-product in the desulphurization of coals by treatment with superheated steam has shown promising insecticidal properties.

Subsequent research has been concentrated upon the steam-volatile fraction of acetone extracts of coal. This has proved to be a complex mixture, but progress has been made towards its characterization by the application of vapour phase chromatography.

I am indebted to Prof. W. D. Evans for advice and encouragement, and to Mr. J. F. Gateley for valuable technical assistance. The award of a D.S.I.R. Research Studentship is gratefully acknowledged.

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Patella aspera Lamarck, New to Norway

During an investigation of the ecology of the genus *Patella* in Norway last summer I had the opportunity of studying large populations of the genus from Western Norway. A close scrutiny of the morphology and habitat, as well as their behaviour in aquaria showed that two species were involved, the common species *P. vulgata* L. and *P. aspera* Lamarck. This is the first record of *P. aspera* for Norway, thus extending its northern limit from north-eastern Scotland[1] to western Norway. The fact that *P. aspera* was observed to spawn in the area investigated shows that the species is an established one in western Norway. *P. aspera* has this year been found from Stavanger (the island Rott) in the south to the islands west of Bergen in the north. Further investigations of distribution northwards will be undertaken this autumn.

In the area investigated the species is common on wave-beaten positions on the outer islands and skerries facing the open sea, but does not penetrate far inshore to sheltered localities. A few specimens were found this summer on the south-western part of Stord, an island outside Hardangerfjord, quite far from the open sea. The species seems to be more restricted in its vertical distribution on the west coast of Norway than it is in Britain. In both these areas it occurs in two separated habitats, partly as a low-water form and partly higher up on the shore in rock pools. In Norway, however, a great part of the low-water forms have been found situated so deep that they are seldom

exposed to air, except during wave-action. They penetrate further down than *P. vulgata*, at least half a meter below mean low-water springs. By far the most specimens seem to be bound to the crust of lithothamnia on the rocks.

The main breeding period for *P. aspera* on the western coast of Norway seems to be July—August—September. For *P. vulgata* it is August to October.

Most of the specimens of *P. aspera* hitherto found have been relatively uniform as to systematic characters. Both in *P. vulgata* and in *P. aspera* the shells show growth rings which are more or less distinct. The radula fraction of *P. aspera* has hitherto been studied on a limited material. It has been found to range between 1·02 and 1·25 in one and the same locality, with a mean value of 1·12. The pluricuspid teeth are of characteristic *aspera* shape, with the outermost cusp as the largest. Some individuals found may possibly represent 'transitional' forms between the two species.

Some experiments have been made on the behaviour of the two *Patella* species in laboratory aquaria. Individuals of the two species taken from the same locality and from the same depth showed a remarkable difference in behaviour when placed on stones and put in aquaria with running sea water. When placed at the lower part of large flat stones rising above the surface, the *vulgata* individuals showed a clear tendency to climb up to the surface, whereas the *aspera* individuals did not show this tendency. On the other hand when the animals were placed on the stones near the surface, the *aspera* individuals showed a tendency to climb downwards, whereas the *vulgata* specimens mostly would stay near the surface. This difference in behaviour suggests a physiological difference in addition to the other differences existing between the two species.

The investigations are being continued and a detailed report will be published later.

I am grateful to Dr. Southward for kindly confirming my identification of *P. aspera*.

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Effective Increase in Nematode Populations by the Addition of Aureomycin

THE usefulness of nematodes of the saprophytic genera *Panagrellus* and *Rhabditis* maintained in mixed culture in corn meal agar for experimental purposes is often limited by severe bacterial contamination. Aureomycin was added to some of these cultures in an attempt to minimize this condition. Casual observations over a period of months seemed to indicate an increased nematode population in the aureomycin plates when compared with the untreated plates. The following experiment was carried out to establish the validity of these observations.

Nematodes from a sponge-starch culture[1] were collected from a Baermann funnel and then allowed to migrate through a column of distilled water. Ten selected adults of the two named genera were then pipetted onto uniform amounts of solidified 1·5 per cent tap water agar in Petri dishes. Aureomycin at concentrations of 1/1,000; 1/10,000; 1/100,000; 1/1,000,000; and 1/10,000,000 were incorporated into the cooling agar and thoroughly mixed prior to

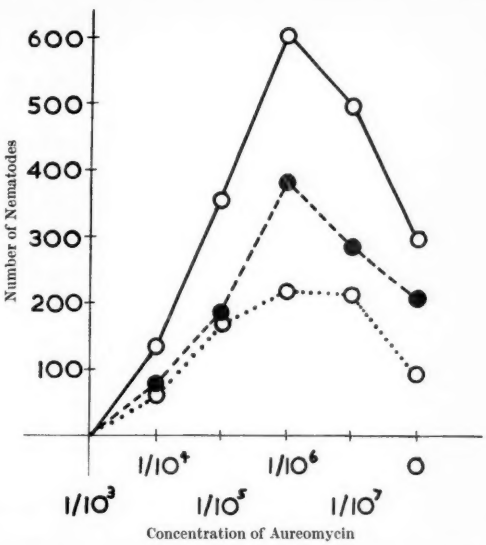

Fig. 1. Nematode populations affected by varying the concentration of aureomycin. —○—, nematodes and eggs; — — ●— — nematodes; · · ○ · ·, eggs.

setting. Each treatment was replicated five times, and an untreated control was also included. Finally, the plates were kept at room temperature for a period of about five weeks. The entire experiment was repeated a second time using the same procedure.

All the plates were marked out with a standard grid consisting of 27 1-cm. squares. The total nematode population was counted in any three randomly selected squares on each dish. This population consisted of eggs, larvæ, and adults found in the agar medium and also on its surface. The results are presented in terms of the number of eggs, the numbers of nematodes (larvæ and adults), and the combined total of nematodes and eggs in the three randomly selected squares. Actual total populations may be estimated by multiplying by a factor of nine for each dish, and by a subsequent factor of five for each treatment.

Statistical analysis of the data indicates that the populations at the lower concentrations of aureomycin are significantly greater than those populations on the untreated dishes. It is also quite clear from the results that the higher concentrations of aureomycin markedly restrict the nematode populations. The maximum increase in the number of nematodes and eggs appears to take place at a concentration of 1/1,000,000.

It is impossible from the results to attribute the increase in nematode populations caused by aureo-

Table 1. MEAN NUMBER OF COUNTED EGGS, NEMATODES, AND EGGS PLUS NEMATODES, FOR EACH TREATMENT. (CONCENTRATIONS OF 1/1,000 AND 1/10,000,000 WERE NOT REPEATED)

Experiment	Concentration of aureomycin	Number of eggs	Number of nematodes	Number of eggs and nematodes
I	Nil	118·0	227·6	345·6
II		63·6	185·0	248·6
Mean		90·8	206·3	297·1
II	1/10,000,000	213·2	284·0	497·2
I		220·4	342·8	563·2
II	1/1,000,000	215·4	426·2	641·6
Mean		217·9	384·5	602·4
I	1/100,000	182·0	222·4	404·4
II		154·6	149·2	303·8
Mean		168·3	185·8	354·1
I	1/10,000	77·8	105·4	183·2
II		37·4	44·0	81·4
Mean		57·6	74·7	132·3
I	1/1,000	0·4	1·4	1·8

mycin to any one biological activity. However, the graph does indicate a uniformity of egg numbers at concentrations of 1/1,000,000 and 1/10,000,000. This tends to suggest a stabilization of reproductive (egg laying) capacity at the two concentrations, showing that an optimum effect has been reached. Further work is definitely necessary for a better understanding of these data.

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Vivipary in *Eleusine Indica* (L) Gaertn

At two localities near Brisbane, plants of *Eleusine indica*, an annual grass, have been observed with inflorescences bearing proliferated spikelets. Such plants were rare and in every instance bore normal as well as proliferated spikelets.

The subtending glumes of the proliferated spikelets were normal but all or some of the lemmas were modified. The modified lemmas varied from near normal to typically leaf-shaped with well-developed sheathing bases and ligules. Usually only the upper lemmas were modified whilst those at the base of the spikelet subtended flowers, which though structurally complete failed to mature. The degree of development of the palea and flower was inversely proportional to the amount by which the lemma was modified. In some proliferated spikelets the lower flowers were replaced with vegetative shoots. The numbers of florets present in samples of normal and proliferated spikelets are summarized in Table 1.

Table 1. Numbers of Florets per Spikelet in Normal and Proliferated Spikelets of *Eleusine indica*

Spikelet	Florets per spikelet											
	0	1	2	3	4	5	6	7	8	9	10	n
normal					1	7	20	6	2	2	2	40
proliferated	3	7	12	10	6	1	1					40

Structurally the proliferated spikelets of *E. indica* closely resemble those of certain viviparous forms of *Festuca ovina*[2]. It is therefore suprising that whereas the proliferated spikelets or bulbils of *F. ovina* grow quite readily into new plants those of *E. indica* seem incapable of further growth if detached from the parent plant. The bulbils in *E. indica* have not been observed to act as propagules in the wild and they have failed to grow when kept under a variety of different cultural conditions. Similar bulbils from inflorescences of *E. corocana* are reported to grow readily if planted[4]. In view of the statement by Gustaffson[3] that no viviparous grass species is diploid, it is interesting to note that *E. indica* is diploid and *E. corocana* is tetraploid[1].

The only other record of vivipary in *E. indica* is from Foochow in China[4]. Both Foochow and Brisbane are at similar latitudes and so have similar day-length régimes. This may be significant as day-length has been shown to be an important factor controlling day-length in *Deschampsia flexuosa*[5]. Further data are required on the occurrence of vivipary in *E. indica* and *E. corocana* to determine whether or not the phenomenon is geographically widespread. Over a period of years in Southern Nigeria, a region of short day-lengths, no proliferated spikelets were observed on either of the above species of *Eleusine* though both species are common there.

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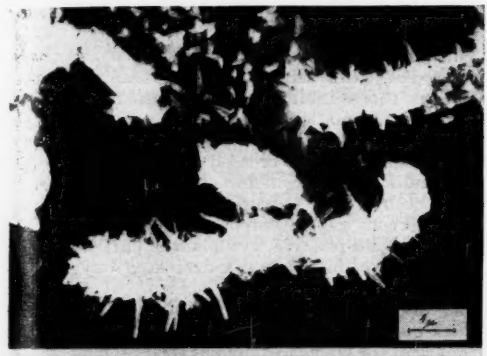
An Electron-Microscopic Study of Spores of Morphological Variants of *Streptomyces erythreus* strains

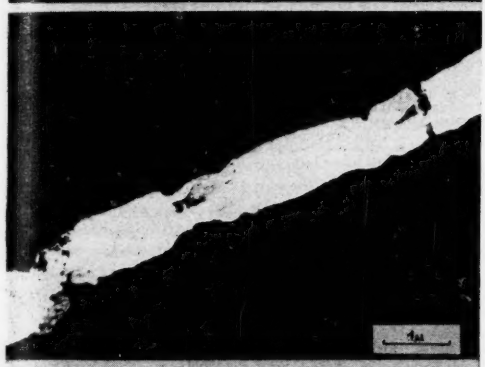
The morphological heterogeneity of lower fungi, based on the different intensity of formation of aerial mycelium and conidia, was found in most of the representatives of the Fungi Imperfecti and was described by Hansen[1] as the 'dual phenomenon'. According to Lindegreen and Andrews[2] the 'dual phenomenon' represents a case of simple heterocaryosis. Heterocaryosis is exhibited by Actinomycetes as well, as shown by Bradley and Lederberg[3].

It was shown indirectly in a previous communication[4] that heterocaryosis was one of the causes of degeneration of the high-production strain *Streptomyces erythreus VUAM*. Electron-microscope studies are herewith described as applied to the spores of morphological variants of the strain *Streptomyces erythreus VUAM* and of two morphologically and productively stable strains *Streptomyces erythreus* No. 58/100 and No. 20-*V*-107. Spores were harvested from a number of morphologically different colonies, obtained by cultivation on complex media[4]. In the case of the rapidly degenerating strain of *Streptomyces erythreus* asporogenic colonies were detected and in addition to them three types of colonies displaying morphological differences of their spores. Two types of colonies characterized by the formation of rich aerial mycelium and by a good production activity, formed chains of oval spiny spores. The spines of the spores were somewhat widened at the base and reached the length of 0·9 μ (cf. Fig. 1). Colonies of the third type, characterized by a poor aerial mycelium and by a low or completely absent production activity, contained in their hyphæ several morphologically different types of spores: chains of solely oval, richly spiny spores (as in Fig. 1), chains of solely rectangular, spineless spores (Fig. 2), and chains comprising both oval and rectangular spores with a minute number of spines (Fig. 3).

Stable strains of *Streptomyces erythreus* forming two morphological types of colonies along with asporogenic types and characterized by the formation of a rich aerial mycelium with a good production activity, produced spores of but one morphological type, that is, oval spores with thick spines. In these strains it was impossible to detect the presence of colonies characterized by a poor aerial mycelium as found in the above-described rapidly degenerating strain *Streptomyces erythreus VUAM*.

The finding that three *Streptomyces erythreus* strains of different origin formed oval spiny spores is not in agreement with the results of Ettlinger





Figs. 1-3. Spores of *Streptomyces erythreus*. 30 days of growth on agar medium No. 3[4], shadowed with gold-palladium

et al.[5], who declare the formation of smooth oval spores as a systematic trait of the species *Streptomyces erythreus*. In this work it was found further that the rapidly degenerating strain of *Streptomyces erythreus* formed several morphologically different spore types. The role of heterocaryosis and/or of other genetic processes during the formation of heterogeneous spores will be studied further.

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Gamma Particles, Nadi-Positive Mitochondria, and Development in the Water Fungi *Blastocladiella* and *Allomyces*

In 1953, it was suggested[1] that genesis of ordinary colourless and orange thalli of *Blastocladiella emersonii* was related to the distribution of a hypothetic cytoplasmic factor, *gamma*, the rate of reproduction of which relative to growth of the protoplast was affected differentially by environmental factors. Later[2], a visible, Nadi-positive, cytoplasmic particle was detected, the distribution of which in the motile swarmers derived from ordinary colourless and orange plants corresponded with that predicted for the hypothetical factor, *gamma*; namely, a low number per spore for orange plants, and a higher number per spore for ordinary colourless plants. The number of these visible particles was, indeed, affected by environmental factors; furthermore, it appeared as if they might be transferred from one cell to another via temporary cytoplasmic bridges formed between orange and colourless motile cells[3]. In addition, studies with a mutant strain of *B. emersonii* which produced only orange plants revealed that: (*a*) the *gamma* content per mutant swarmer was the same as the *gamma* content of the wild-type orange swarmers from orange plants[2]; (*b*) such mutant, orange plants displayed a respiratory lesion in the tricarboxylic acid cycle; namely, they lacked the oxidative activity associated with α-ketoglutarate dehydrogenase normally found in the wild type[4,5]. In recent years, it has begun to appear[6] as if cytoplasmic control of differentiation of orange and colourless cells in the close relative *Allomyces* may also be related to the distribution of similar, Nadi-positive particles, particularly in view of the recent demonstration[9] of a parallel sort of differential distribution of Nadi-positive 'mitochondria' in the unicellular orange and colourless sex organs of this fungus.

With the correlation between severe reduction in oxidative activity and decreased numbers of Nadi-positive, cytoplasmic particles in *Blastocladiella* in mind, it seemed likely[8,9] that orange and colourless plants of *B. emersonii* would respond differently to

Table 1. Growth about 20° C. of Swarmers from Orange, Orange Colourless are Resistant-Sporangial Plants of *B. emersonii* in 50 ml. of Difco 'Cantino Pyg Broth' containing Bromcresol Purple as *p*H Indicator

	Anaerobic tubes Days after inoculation				Aerobic controls			
	2	3	4	7	2	3	4	7
Inoculum source	Volume of growth (arbitrary scale)							
Spore suspension derived from a single resistant-sporangine plant	0	0	0	1	0	1	2	3
" "	0	0	0	½	½	1	2	3
" "	0	0	0	0	1	1½	2	3
" "	0	0	0	0	1	2	3	4
Spore suspension derived from a single ordinary colourless plant	0	0	0	1	0	1	2	3
" "	0	0	0	1	0	1	1½	3
" "	0	0	½	1	0	1	2	3
" "	0	0	0	1	1	1½	2	3½
" "	0	0	0	½	1	1	2	3
Spore suspension derived from a single orange plant	0	1	1½	2	0	1	2	3
" "	½	1	1½	2	0	1	2	3
" "	0	½	½	1½	1	2	2½	3
" "	0	1	2	2½	0	1	2	3
" "	½	1	1	2	½	1	2	2½

The anaerobic tubes were fitted with alkaline pyrogallol plugs and small, methylene blue indicator tubes. Growth tubes were calibrated (arbitrary scale) for measurement of packed volumes of plants. The volume-to-plants ratio in these experiments was such that *p*H changes were not significant until the seventh day

anaerobic conditions. By the same token, it followed that the hermaphroditic, wild type and the orange, male (mutant) strains[7] of *Allomyces macrogynous* should also exhibit a differential response to reduced oxygen tensions. Therefore, several experiments were carried out to test this hypothesis.

In *Blastocladiella*, growth under reduced oxygen tensions of the first few generations of progeny derived from orange swarmers (which, at the start, possess relatively few Nadi-positive *gamma* articles) begins to approach that obtained under normal, aerobic conditions; but, growth under such reduced oxygen tensions of the progeny from ordinary colourless swarmers (and resistant-sporangial swarmers, which also possess the larger number of *gamma* particles) is almost nil (Table 1). In *Allomyces*, too, anærobiosis has a anaerobic conditions. By the same token, it followed differential effect. Under aerobic conditions on solid media, the orange mutant always displays a distinct lag in its growth relative to the wild type; furthermore, once linear growth-rates become established, the wild type grows more rapidly (under our conditions, a 15 per cent increase at 27° C.) than the orange, male mutant (Fig. 1, top). But, under reduced oxygen tensions (for example, under mineral oil, and under tank nitrogen where the partial pressure of oxygen is about 1 mm. of mercury), this differential is not maintained and both strains grow at comparable rates (Fig., bottom).

Thus, in both *B. emersonii* and *A. macrogynous*, decreased oxygen tensions exert a minimum inhibitory effect on those thalli which possess both a high potential for formation of pigmented cells (γ-carotene) and a small number of Nadi-postive, cytoplasmic particles; conversely, decreased oxygen tensions exert a maximum inhibitory effect on those thalli which possess both a high potential for formation of non-pigmented cells (no γ-carotene detectable) and a large number of Nadi-positive, cytoplasmic particles.

Furthermore, in *Allomyces* decreased oxygen tensions favour an increase in the incidence of the orange (male) cells, particularly on peptone media (*PYG*) but also on yeast-starch media (*YpSs*); conversely, the incidence of the colourless (female) cells, which possess larger numbers of Nadi-positive particles than the orange ones, is reduced (Table 2).

Table 2. Incidence of Male and Female Sex Organs in 12–30 Day Cultures of Wild-Type *Allomyces macrogynous* Grown under Aerobic and Semi-Anaerobic Conditions at 26° C. on Difco 'Emerson YpSs Agar' and Difco 'Cantino Pyg Agar'

Medium	Aerobic			Semi-anaerobic† (mineral oil)		
	male	female	per cent male/female	male	female	per cent male/female
'YpSs'	316	293	52	324	235	58
'PYG'*	241	342	41	454	241	70

Counts were made on numerous random samples; representative data are shown above.

* As compared to medium 'YpSs', medium 'PYG' tends to promote femaleness in *Allomyces* under aerobic conditions, and this is reflected microscopically in chains of two or more gametangia in which females predominate. Reduced oxygen tensions reverse this situation whereby the orange, male sex organs become dominant.

† Counts were made just below the oil-air interface; further down in the more deeply submerged areas of the slant, mycelia are completely sterile. It seems clear that a critical, but as yet undefined range of oxygen tensions is involved in the phenomenon.

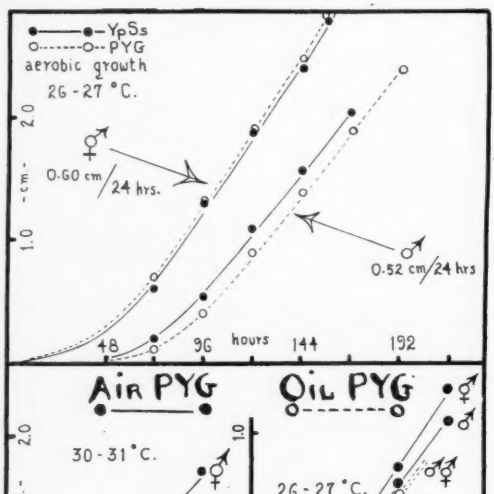

Fig. 1. The growth of wild-type and male (mutant) strains of *Allomyces macrogynous* under air and reduced oxygen tensions at 27° C. and 31° C. on 'PYG' and 'YpSs' media. Top: representative growth data for both strains under air at 26–27° C. For medium 'PYG', growth was measured by recording linear progress of the mycelial front along horizontal culture tubes; for medium 'YpSs', it was measured by recording radial progress of the mycelial front in Petri dishes. Bottom: representative comparative growth data for both strains under air and under semi-anaerobic conditions (for example, under mineral oil) at 26–27 C. and 30–31° C. In this case, zero time represents a point three days after incoculation when growth curves had become linear. Growth was measured by recording linear progress of the mycelial front along vertical culture tubes (slants)

These results provide evidence that: (*a*) the oxygen requirement for differentiation of sex organs in *Allomyces* is greater than that needed for vegetative growth; (*b*) that the oxygen requirement for development of orange cells (male sex organs) in both *Allomyces* and *Blastocladiella* is less than that needed for development of their colourless counterparts (female sex organs); and (*c*), the Nadi-positive *gamma* particles in *Blastocladiella* and the Nadi-positive 'mitochondria' in *Allomyces* may well be implicated, directly or indirectly, in the metabolic basis for sexual differentiation and carotene synthesis in the Blastocladiaceae.

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PATHOLOGY

Growth Inhibition of Mouse Ascites Tumour Cells by Powdered Tragacanth (Tragacanthæ Pulvis, B.P.)

DURING experiments designed to explore possible short-term effects of 'Busulphan' (1 : 4-dimethanesulphonyloxybutane or 'Myleran') on mouse ascites tumour cells, the drug was injected intraperitoneally in suspension in arachis oil or in an aqueous solution of compound powder of tragacanth into $C+$ male mice bearing the Landschutz ascites tumour. Neither the arachis oil suspension of 'Busulphan' nor the oil alone produced any inhibition of tumour cell growth. However, both the suspension of 'Busulphan' in compound powder of tragacanth solution and the tragacanth solution alone totally inhibited the tumour, as judged by absence of ascites tumour cells (and of solid tumour) seven days after injection of 25 mgm. of the compound in $\frac{1}{2}$ c.c. water or saline. The same result was obtained after a lower dose of the powder (2·5 mgm. per mouse), in all cases injected one day after the inoculation of the tumour cells. A dose of 10 mgm. per mouse in a 3-day-old tumour prolonged longevity two-fold (longevity in days for treated: control tumour-bearing animals = 33 : 16). Of other tumours tested, total inhibition was also obtained at a dose of 2·5 mgm. per mouse seven days after inoculation of the *Bp*8 ascites tumour in *C3H* mice; but no effect was observed by intravenous or subcutaneous administration to $C+$ mice bearing a solid subcutaneous implant of the $C+$ leucæmia. (This arose in the Chester Beatty Research Institute as a spontaneous lymphocytic leucæmia in $C+$ mice.)

The compound powder of tragacanth had been compounded for pharmaceutical purposes from four components in the proportions: powdered tragacanth 1·5, powdered acacia 2·0, maize starch 2·0 and sucrose 4·5. Parallel tests of 2·5 mgm. of the compound (in 0·5 c.c. saline) per mouse and of proportionate amounts of these four components showed that at these concentrations the tragacanth powder was the growth inhibitory agent. Most of the recent tests have been carried out with this sample of tragacanth powder, that is, a sample of Persian origin, B.D.H., B.P. No. 2.

For example, by intraperitoneal injection into the Landschutz ascites tumour in $C+$ male mice, 3 mgm. tragacanth powder given to mice bearing a 3-day-old tumour increased the longevity of the mice in the ratio 33 : 18; and in another experiment 2 mgm. of the powder tested against 3-day-old tumours gave a longevity increase of 54 : 19. 5 mgm. powder per animal in a 7-day-old tumour resulted in a longevity increase of 23 : 18 or, on the basis of a total tumour cell count 14 days after the tumour cell inoculation, in a ratio, number of ascites tumour cells in control animals: number in treated animals (that is, $C/T_{(14)}$) of 32. Similar results have been obtained for the *Bp*8 ascites tumour in *C3H* male mice, the Crocker ascites tumour in stock mice, and the $C+$ leucæmia (ascites) treated intraperitoneally in $C+$ mice. However, administration of the tragacanth in the drinking water (68 mgm. per mouse, that is, about 10 mgm. daily for 7 days) produced no inhibition of the Landschutz ascites tumour (cells counted 7 days after inoculation, that is $C/T_{(7)} = 1{\cdot}09$).

No inhibitory action has been detected in the solid tumour tested (Crocker tumour in stock mice) when 52 mgm. tragacanth powder per mouse was taken freely in drinking water over an 8-day period, or when 160 mgm. was given by forced feeding in the same period. At the end of the latter experiment the animals were in poor condition, although no ill-effect was observed after tragacanth administered in drinking water. Administered intraperitoneally, larger doses of the agent are tolerated in animals bearing older tumours.

Tests have been initiated using tragacanth samples of different commercial grades. Their inhibitory activity varies with the sample and the first results suggest that Indian tragacanth powders (gum Karaya, from *Sterculia urens*) and Persian flake powders (from *Astragalus gummifer* Labillardiere) are non-inhibitory at non-toxic doses; while Persian ribbon powders, which are from *A. gummifer* from a different habitat are inhibitory at quite low, non-toxic doses, for example, 2 mgm. per mouse gave a $C/T_{(7)}$ of 60 to infinity.

It is of interest that a gum tragacanth was tested against the Ehrlich ascites tumour in mice by Oettel and Wilhelm[1] and was reported to have no effect on tumour size, average body weight or average survival time for treated animals compared with controls, at a dose of 25 mgm. per kgm. body-weight, that is, five daily doses of 5 mgm. per kgm., commencing injections 2 hr. after tumour inoculation. At a dose of 250 mgm. per kgm. body-weight (50 mgm. × 5 daily doses) there were small reductions in treated animals in all three quantities measured, that is, a ratio for treated to control mice of 3·5 : 4·7 for average ascites tumour weight (gm.), of +0·5 : +1·2 for average body-weight change (gm.), and 12·5 : 15·7 for average survival time (days), suggesting a toxic effect at this dose level. A single injection of 1–2 mgm. per mouse in our experiments (normally to mice weighing initially 21–24 gm.) is intermediate between the doses tested by Oettel and Wilhelm and has a significant tumour-inhibitory effect even on a 3-day-old tumour. It is possible, therefore, that the sample of gum tragacanth used by these authors is similar in origin to those found non-inhibitory in our tests.

I am grateful to Prof. A. Haddow for his interest in this work and to Mr. E. Mayhew and Mr. C. Smith for their skilled assistance. Messrs. Robert Stewart and Sons (London) have kindly provided different grades of tragacanth powder.

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Aug. 21.

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BACTERIOLOGY

Function of Carotenoids in Photosynthetic Bacteria

A RELATION between photosensitivity and the loss of carotenoid pigments has been observed in *Rhodopseudomonas*[1], *Chlorella*[2], *Chromatium*[3], *Chlamydomonas*[4], *Rhodospirillum*[5], *Corynebacterium*[6] and *Zea mays*[7]. Sistrom *et al.* have suggested that photosensitization in the carotenoidless mutant of *Rhodopseudomonas spheroides* '. . . is a specific consequence

of the replacement of carotenoid pigments with a colourless polyene.' and that '. . . the primary function of carotenoid pigments in phototrophs is to act as chemical buffers against photo-oxidation of other cell constituents by chlorophyll, thus conferring a high degree of immunity to endogenous photosensitization.'[8] The carotenoid may carry out this buffering function by acting as a preferentially photo-oxidizable substrate. The effectiveness of such a photochemical buffering system would depend on the existence of an enzymatic mechanism which could reduce the photo-oxidized carotenoid. Inhibition of this enzymatic reduction should result in photosensitization. Accordingly, the effect of low temperature on the photosensitivity of wild type *R. spheroides* (2.4.1) and *Rhodospirillum rubrum* was investigated.

The protocol for a typical experiment (Exp. 2, Fig. 1) is as follows. *R. spheroides* was grown photosynthetically in a stab culture[9]. A small amount of growth was inoculated into a 50-ml. Florence flask filled to the neck with modified Hutner's medium[9]. This was incubated in the light at 30°C. until the optical density at 680 mμ had reached 1·5. In order to obtain actively growing cells in the logarithmic phase of growth, 0·4 ml. of the Florence flask culture were inoculated into a large test tube (2·8 × 20 cm.) containing 40 ml. of modified Hutner's medium. The tube was fitted with an aerating and sampling device, placed about 1 cm. from the entrance window in a glass water bath maintained at 30°C. and bubbled continuously with a mixture of 95 per cent nitrogen and 5 per cent carbon dioxide. The culture was illuminated with two 300-watt photo-flood lamps and the intensity was adjusted to 600 foot-candles. When the culture had reached an optical density of 0·255 and a specific chlorophyll[10] of 0·86, 40 ml. of suspending medium (5 ml./1. modified Hutner's base and 0·02 *M* potassium phosphate buffer, *p*H 6·8) were inoculated to a density of $1{\cdot}8 \times 10^5$ cells per ml. The suspension was placed in a water bath maintained at 1°C. and was aerated continuously with a mixture of 95 per cent air and 5 per cent carbon dioxide. The light intensity was adjusted to 1,500 foot-candles. A similar tube was aerated at 1°C. in the dark. Samples were taken for plating before irradiation and after 8 hours.

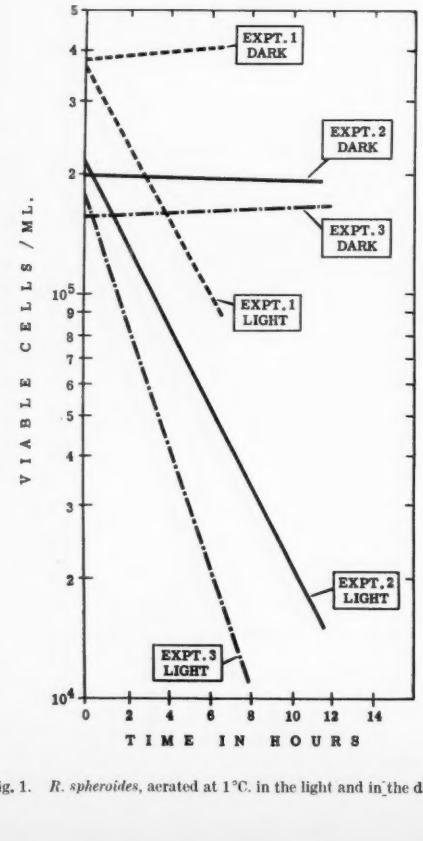

Fig. 1. *R. spheroides*, aerated at 1°C. in the light and in the dark.

Fig. 1 shows that when *R. spheroides* is aerobically irradiated at 1°C. there is definite photo-killing. Control cells aerated at 1°C. in the dark do not show this effect. Since counts were made only at the beginning and end of the experimental period it was not possible to determine if the killing was preceded by a lag period.

There was no destruction of chlorophyll during the irradiation. This is consistent with earlier results[10] which showed that chlorophyll destruction in the carotenoidless mutant of *R. spheroides* was inhibited at low temperatures.

Essentially the same behaviour was noted with cultures of *R. rubrum*.

The inhibition by low temperature of the protective function of the carotenoids brings a number of questions to mind: (1) Can an oxycarotenoid reductase be isolated? (2) What is the nature of the hydrogen donor for the reduction? (3) Could this reduction play a role in the energetics of photosynthesis?

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Function of Carotenoid Pigments in Non-Photosynthetic Bacteria

It has been shown by several workers that the carotenes of photosynthetic bacteria protect the organisms against a lethel photodynamic reaction in which intracellular bacteriochlorophyll is the photosensitizer[1,2]. It has been suggested that the carotenes found in many non-photosynthetic bacteria may have the same protective function in these organisms[1]. Here, some other compound would be the photosensitizer in place of bacteriochlorophyll.

If this suggestion be true, carotene-less mutants of normally pigmented bacteria will be killed in the presence of light and air. This has been tested by

Kunisawa and Stanier using a colourless mutant of *Corynebacterium poinsettiæ*[3]. Although it was found that the carotenes of the wild-type protected the cells against photodynamic killing, the colourless mutant was killed only if an exogenous photosensitizer were added. We have obtained similar results using a carotene-less mutant of *Sarcina lutea*. We were unable to show any killing of the mutant, in the absence of added photosensitizer, after exposure for 4 hr. to light from either tungsten lamps (3,000 ft.-candles) or fluorescent lamps (1,200 ft.-candles). However, in the presence of toluidine blue and light and air, the colourless mutant is rapidly killed, while the wild-type is unaffected, as is the mutant in the absence of either light or oxygen.

At first sight these experiments suggest that the protection afforded by carotenes is ecologically significant only in the photosynthetic bacteria. However, the experiments with the photosynthetic bacteria and those with the non-photosynthetic bacteria are not really comparable. The absorption maximum of bacteriochlorophyll at 850 mμ is near the emission maximum of tungsten; on the other hand, it is unlikely that an intracellular photosensitizer in non-photosynthetic bacteria, if it existed, would absorb strongly above 500 mμ, but at wavelengths smaller than this the emission of tungsten is relatively very low.

These considerations led us to compare the effect of direct sunlight on the wild-type and carotene-less mutant of *S. lutea*. Fig. 1 shows the results of a typical experiment. It can be seen that after an exposure of 2 hr., more than 99 per cent of the mutant cells have been killed; in the same period the pigmented cells have not been affected. The mutant cells are not killed in the absence of oxygen, thus demonstrating that the killing by direct sunlight is photodynamic (Fig. 1).

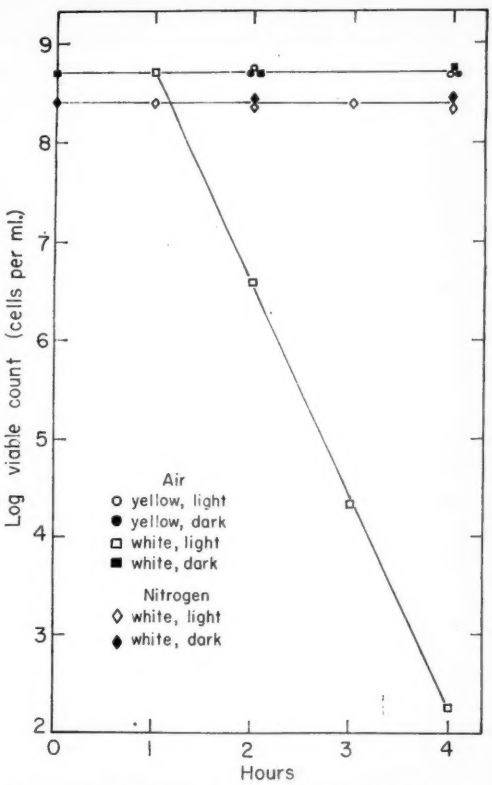

Fig. 1. Effect of exposure of colourless and pigmented *S. lutea* to sunlight, and effect of exposing the colourless mutant of *S. lutea* to the Sun in an atmosphere of nitrogen. Light intensities were; 0 time, 12,000 ft.-candles, 2 hr. 10,000 ft.-candles, 4 hr., 5,000 ft.-candles. Light intensity in this and the other experiments discussed was measured with a Weston illumination meter, model 756, which is standardized by tungsten filament light. The cells were suspended in a buffer containing, per litre, 50 ml. of 1 *M* potassium phosphate buffer (*p*H 7·0), 20 ml. of concentrated mineral base, 1 mgm. of nicotinic acid, 0·5 mgm. of thiamine, and 0·01 mgm. of biotin. Air or nitrogen was bubbled through each cell suspension during the experiment

From these experiments we conclude that the carotenes of non-photosynthetic bacteria do protect the cells against a lethal photodynamic action which can occur in the absence of exogenous photosensitizer and in naturally occurring light fields. The lethal action of visible light is not limited to carotene-less mutants of pigmented bacteria, but can occur in normally carotene-free bacteria[4]. It should then be possible, if a primary function of carotenes is to protect against photodynamic killing, to understand the major features of the distribution of these pigments in bacteria: on the whole, we expect organisms that are normally exposed to both light and air to contain carotenoid pigments, and organisms that are not exposed to these agents during their usual life-histories not to contain them. This has been pointed out previously[1], but in view of the experiments reported here it may be worth while to make more precise descriptions of the kinds of bacteria in which one would expect selection to have favoured the development of carotenoid pigments, and to compare these with the known carotenogenic bacteria.

Many aquatic bacteria are certainly exposed to both light and air. It has been noted that a majority of aquatic bacteria are pigmented; many of these pigmented forms are probably carotenogenic[5]. Members of the *Flavobacteria*, an aquatic genus, are known to contain carotenes[6].

The situation is more complex in the soil. It seems likely that most aerobic terrestial bacteria will not be exposed to high light intensities while in the soil. Thus, motile terrestial bacteria, which can presumably disperse themselves through the soil, would never be subject to photodynamic killing, and pseudomonads, the most important representatives of this kind of bacteria, are with few exceptions non-carotenogenic. Aerial dispersion will subject bacteria to very intense light fields. Spores are probably resistent to photodynamic killing and carotenes are seldom, if ever, found in the bacilli. Asporogenous bacteria that are aerially dispersed will, on our hypothesis, contain carotenes. This group will include the Mycobacteria and Corynebacteria, both of which are notable for their carotenoid pigments. A more complete account of these experiments is to be published.

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An Absolute Requirement for 'Iron Transport Factors' by *Microbacterium lacticum* 8181

We recently published a report on the growth stimulation of *Microbacterium* sp. by 'iron transport factors' such as ferrichrome, coprogen and the terregens factor[1]. These factors acted by decreasing the duration of the lag-phase of growth and were by no means essential. The culture resembled *M. lacticum* more closely than *M. flavum*, but possessed characteristics of both species. We felt it of interest to determine whether authentic species of *Microbacterium* were also stimulated by such factors.

M. lacticum 8180, *M. lacticum* 8181 and *M. flavum* 10340 were obtained from the American Type Culture Collection and grown on slants containing Difco nutrient agar plus 1 per cent Difco yeast extract. After 48-hr. incubation at 37°C., the cells were harvested and washed twice with 0·02 *M* dipotassium hydrogen phosphate. The remainder of the procedure and the composition of the basal medium were as previously described[1]. The 'iron transport factors', ferrichrome[2] and coprogen[3], were used 100 (mμgm./ml.

Of greatest interest was the result observed with *M. lacticum* 8181. As shown in Fig. 1, this strain has an absolute requirement for ferrichrome or coprogen. *M. lacticum* 8180 resembled our isolate[1] in that 'iron transport compounds' merely stimulated lag-phase growth. The growth of the *M. flavum* culture was not influenced by these compounds.

M. lacticum 8181 would appear to be a better organism for assay of 'iron transport factors' than those previously described. Its use is much less laborious and time-consuming than that of *Pilobolus kleinii*[4]. Also, *M. lacticum* requires only half of the incubation time and gives higher optical density values than the recommended *Arthrobacter* cultures[5].

We wish to thank Dr. J. B. Neilands of the University of California and Dr. B. L. Hutchings of the Lederle Laboratories for the samples of ferrichrome and coprogen, respectively. Acknowledgment is also made to Mrs. R. Marsh for technical assistance.

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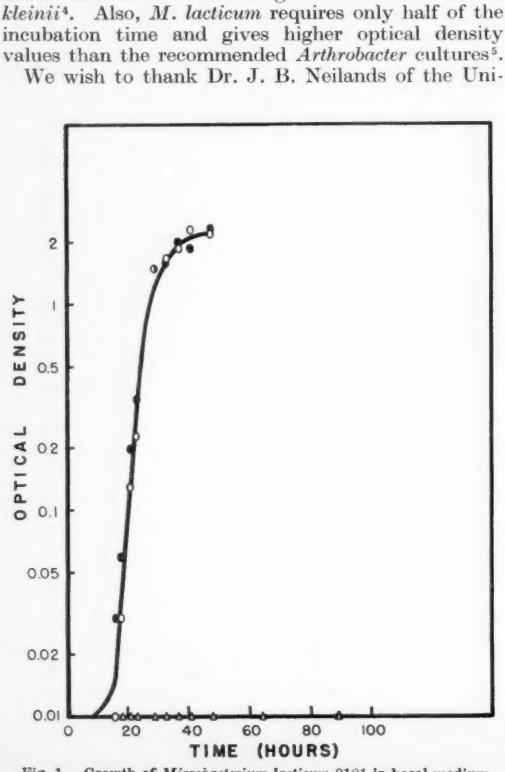

Fig. 1. Growth of *Microbacterium lacticum* 8181 in basal medium (▲), in basal plus 100 mμgm. ferrichrome per ml. (●) and in basal plus 100 mμgm. coprogen per ml. (○).

Selective Inhibition of Ribonucleic Acid Synthesis in *Staphylococcus aureus* by Vancomycin

Vancomycin, an antibiotic obtained from *Streptomyces orientalis*, is effective against a number of growing bacteria, particularly those which are Gram-positive. Mutation to resistance towards this compound is of a very low order, and the antibiotic has been shown to be clinically effective against strains of *Staphylococcus aureus* which are resistant to the more common antibiotics.

During investigations on the mode of action of vancomycin (free base) on *S. aureus* it was found that after its addition to a logarithmic-phase culture both nephelos and optical density readings continued to increase for a period of about 60 min., although at a reduced rate. During this same time-interval total cell counts remained constant and plate counts rapidly decreased. Therefore, during the initial stages of its activity, vancomycin inhibits cellular division and yet allows protoplasmic synthesis to continue. No reversal of the action of the antibiotic was obtained by washing vancomycin-treated cells in water or various buffers and no ameliorative effect was found when various concentrations of certain purines, pyrimidines, peptides and metallic ions were added to the growth medium prior to the addition of the vancomycin.

Cells grown in brain heart infusion (Difco) for 21 hr. at 37° C. were harvested by centrifugation. These cells were resuspended in a smaller quantity of fresh medium and incubated until nephelos readings showed a constant increase. The culture was then divided into two parts, to one of which was added vancomycin to a final concentration of 100 μgm./ml. (about 37 μgm./mgm. cell dry weight). The other portion served as a control. Five ml. amounts of culture were removed at various time intervals for analysis. Nephelos readings were taken with a photonephelometer. Protein was determined by the Folin–Ciocalteu method, deoxyribonucleic acid by the diphenylamine reaction after hot perchloric acid extraction, and total nucleic acid by spectrophotometry at 260 mμ and the use of an

Table 1. Effect of Vancomycin on Growth and the Synthesis of Protein, Deoxyribonucleic Acid and Ribonucleic Acid in *Staphylococcus Aureus*

Final concentration of vancomycin (μgm./ml.)	Time after addition of vancomycin (min.)	Protoplasmic growth (nephelos units)	Protein (mgm./5 ml. culture)	Deoxyribonucleic acid (mgm./5 ml. culture)	Ribonucleic acid (mgm./5 ml. culture)
0	0	70·5	8·40	0·10	0·93
	30	76·5	9·20	0·12	1·03
	60	85·5	10·16	0·17	1·13
120	120	87·0	10·56	0·17	1·19
100	0	70·5	8·40	0·11	0·93
	30	76·5	9·20	0·12	0·95
	60	80·0	10·16	0·16	0·95
	120	78·5	9·76	0·16	0·88

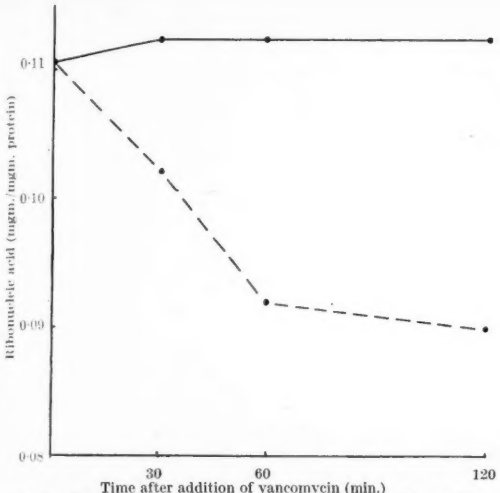

Fig. 1. Effect of vancomycin on the synthesis of ribonucleic acid by *S. aureus*, ———, Control ; — — —, plus vancomycin

extinction coefficient of 28[1]. Ribonucleic acid was calculated as the difference between total nucleic acid and deoxyribonucleic acid (Table 1).

During the time period after the addition of the antibiotic when the nephelos readings were still rising there was little or no difference in the rate of synthesis of either protein or deoxyribonucleic acid when compared with the control. There was, however, a complete inhibition of synthesis of ribonucleic acid. (Fig. 1.)

The role of nucleic acid in protein synthesis is highly controversial. Among the workers who believe that the two are mandatorily related are those who are of the opinion that net synthesis of ribonucleic acid is a requisite for protein production. The present communication, however, would appear to constitute evidence that under the experimental conditions used net synthesis of ribonucleic acid is not an absolute requirement for the biosynthesis of protein. Nevertheless, it cannot be concluded that synthesis of ribonucleic acid and protein are not interrelated because the former still remaining intact in vancomycin-treated cells may be able to direct protein formation for a short interval of time. Certain precursors of ribonucleic acid may even carry on this synthesis in a manner somewhat analogous to Gale's 'incorporation factors'[2].

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Influence of DL-beta-Phenylserine on the Synthesis of Proteins in *Escherichia coli*

THE influence of phenylserine, the structural analogue of phenylalanine, on the synthesis of proteins has been studied from three points of view : (*a*) the effect on the incorporation of amino-acids, (*b*) the effect on the formation of adaptive enzyme (beta-galactosidase) and (*c*) the effect on growth.

All experiments described here were performed with a strain of *Escherichia coli* requiring serine-plus-glycine and with strain *M*-200 requiring methionine of the same species. Cultivation was carried out on a shaker at 30°C. using the synthetic medium of ref. 1. Glycine, DL-serine and DL-methionine were added to a final concentration of 2×10^{-3} *M* of L-amino acid.

Incorporation of amino-acids was carried out with cells from the exponential phase of growth (when the culture contained about 0·450 mgm. dry weight of bacteria per ml.) which had starved prior to incubation to exhaust intracellular amino-acids[2]. Extraction of proteins and preparation of samples for radioactivity measurements were carried out by Mandelstam's method[2]. Radioactivity was measured in an infinitely thin layer on an end-window (1·12 mgm./cm.^{2}) Geiger-Müller counter.

Beta-galactosidase activity was determined in toluene-treated cells at 37°C. by Rickenberg and Lester's assay method[3] with *o*-nitrophenol-beta-D-galactoside as substrate.

It was found (Fig. 1) that phenylserine in a medium containing amino-acids decreased the incorporation of alanine-2-^{14}C, while in a medium without glycine and serine the presence of phenylserine had a stimulatory effect. Incorporation of alanine was the same as in the presence of amino-acids and was many times higher than in the medium without amino-acids and without phenylserine. Similar results were obtained with the incorporation of methionine-^{35}S.

Estimating the synthesis of beta-galactosidase gave analogous results. In the medium containing amino-acids, enzyme synthesis was inhibited by phenylserine almost completely, whereas in the absence of glycine and serine the synthesis of the enzyme was stimulated, so that the synthesis of beta-galactosidase was about 2–3 times higher than in the medium with amino-acids and phenylserine.

In all cases when phenylserine was present in the medium, no growth was observed during the first two hours, when the incorporation and the synthesis of beta-galactosidase were studied. The first signs of growth were registrated only after four hours in the amino-acid medium.

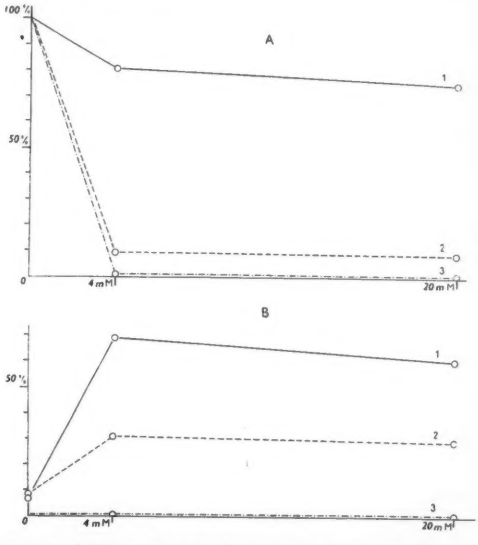

Fig. 1. Influence of phenylserine on the incorporation of alanine-2-^{14}C (1), on the formation of beta-galactosidase (2), on growth (3) : (*A*) in the presence of glycine and serine ; (*B*) without glycine and serine.
Abscissa : concentration of phenylserine. Ordinate : percentage of incorporation, of formation of the enzyme and of growth

An attempt was made to ascertain whether phenylserine acted as a whole molecule or whether the stimulation of proteosynthesis was caused by some of its enzymatic decomposition products. Glycine was considered as the most likely product of the splitting of phenylserine. It was found in the experiments with phenylserine-1-^{14}C that this analogue was incorporated into the proteins of the strain of *E. coli* requiring serine-plus-glycine approximately in the same quantity both in the medium with glycine and serine and that without these amino-acids. It was demonstrated in further experiments that phenylserine is broken down by this mutant to benzaldehyde and glycine. The amount of glycine formed is less than 1 per cent of phenylserine during 2 hr. at 30°C. This was shown chromatographically with autoradiographic detection. It is believed, therefore, that the increased incorporation of amino-acids by this strain is caused by the liberation of glycine from phenylserine. This view is supported by analogous experiments with the strain requiring methionine, in which it was demonstrated that incorporation of alanine-2-^{14}C in the presence of phenylserine was not stimulated by the analogue and was the same as in the medium without amino-acids and phenylserine.

These results lead to the conclusion that the mechanism of the synthesis of proteins and its inhibition is strongly influenced by the analogue as well as by the quantity of utilizable amino-acids. The most sensitive step to the presence of the analogue seems to be growth during the first two hours. The synthesis of beta-galactosidase seems to be less affected. The incorporation of amino-acids is influenced only slightly.

Stimulation by phenylserine of beta-galactosidase synthesis in deficient medium is difficult to explain.

Possibly, if the quantity of essential amino-acid is very small, as in the case of glycine produced from phenylserine, only certain proteins are synthesized, especially in the case where the multiplication of cells is markedly reduced by the analogue.

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VIROLOGY

A Virus Disease of *Meloidogyne incognita incognita*, the Southern Root Knot Nematode

This communication reports the presence of a virus in the plant parasitic nematode *Meloidogyne incognita incognita* (Kofoid and White) Chitwood.

While *M. incognita incognita* larvæ in the proximity of a tomato seedling normally move to the roots within an hour[1], in one experiment all the larvæ were extremely sluggish. Usually entrance of the larvæ into the host is accompanied by gall formation, but none of these larvæ formed any galls. A nematode disease was suspected. Microscopic examination of the larvæ failed to indicate any fungi; but the larvæ appeared to be highly vacuolated or filled with unusually prominent oil-like globules. To maintain the disease ½ ml. of a suspension of sluggish nematodes was added to a Petri dish with several thousand surface-sterilized nematode eggs in 30 ml. triple strength modified Heller's solution adjusted to *p*H 6·5[1,2]. As a control, some of the eggs were not inoculated. The larvæ that hatched from the eggs to which the sluggish nematodes had been added were slow and jerky in their movements. The posterior portion of their bodies appeared to lose power of locomotion first. Many of them died soon after emerging whereas the control larvæ were normal. Upon death the infected larvæ were in various shapes, but usually not in the elongated shape of a relaxed nematode. The disease was maintained for the next three weeks by transferring six diseased larvæ every week into a dish of healthy, surface-sterilized eggs.

Having found that the disease could be transmitted, we tested the possibility that it was caused by a virus. The contents of several dishes containing diseased larvæ were pooled and filtered through two filter sheets of type *ST*–3 and size *L*–6 in a Seitz filter, which had been pretreated by filtration of 50 ml. ½ per cent neopeptone solution containing 0·05 per cent 'Antifoam *A*' (Dow) and then sterile distilled water. Aliquots of filtrate added to sterile nutrient agar medium failed to indicate the presence of any bacteria or visible micro-organism. In addition, two experiments were conducted whereby a bacterial suspension of *Corynebacterium poinsettiae* Starr and Pirone, which averages 0·3–0·8 by 1·0–3·0 μ in size, was added to the suspension of diseased nematodes prior to filtration. Two loopfuls of each of ten 2-ml. aliquot samples of filtrate were transferred to nutrient broth but no growth was visible. However, growth of the bacteria was observed in comparable controls. Another aliquot was added to a batch of healthy surface-sterilized *M. incognita incognita* eggs in triple strength modified Heller's solution adjusted to *p*H 6·5; the remainder of the nematode eggs were maintained as controls. Larvæ from both sets of eggs were placed, one week later, in the vicinity (3 mm. from roots) of tomato seedlings growing on an agar medium. The control larvæ reached the roots within 1 hr.; the other larvæ either did not reach the roots at all or required more than 3 hr. to do so. A Seitz filtrate prepared from the suspension in which the latter nematodes had emerged spread the disease to another sample of healthy eggs. These observations indicate that the infectious agent could pass through a Seitz filter and that its virulence was not lost by serial passage.

The fact that this disease of *M. incognita incognita* larvæ is transmitted by a filterable agent indicates a virus as the cause. Investigations are under way to characterize further the filterable agent, to determine its host range, and to employ its disease-producing power as a practical control of nematodes.

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